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This chapter will examine the problems and facts pertaining /1* to developing a human living environment by biological methods, i.e., utilizing the appropriate functions of atrophic and heterotrophic organisms.

Biological methods of life support are usually connected with the longest periods of autonomous existence of spacecraft crews.

Therefore, the problem of the human life support systems over a period of many years in an artificially produced environment brings to the foreground the general problems of human ecology, in addition to particular problems. This problem requires careful examination and further development of the basic concepts of the contemporary theory of the human living environment in a wide sense, and also a critical analysis of those practical models of this environment, which we shall use for standardizing the atmosphere, the food supply, and the quality of drinking water in spacecraft. The possibility is not excluded that our present day concepts regarding the basic necessary elements in the environment may be incomplete, and insufficient for the biological requirements of humans under conditions of prolonged autonomy.

*Numbers in the margin indicate pagination of original foreign text.

The literature has already made the first attempts to examine the basic parameters of the gas medium for prolonged space flights, based on the concept that the living environment must maintain the human organism under conditions of periodic functional loads, which are necessary to maintain the initial level of the organism reactivity [24, 25].

The incompleteness of our concepts regarding the basic factors of the human environment is particularly apparent when viewed from the historical development of vitamins and vitamin deficiencies, particularly the discoveries constantly being made regarding the new, biologically effective foods which have vitamin and vitamin-like action. The concept of the atmosphere as a solution of oxygen and carbon dioxide in an inert gas does not reflect the complexity of the actual composition of the biogenic atmosphere of the Earth, which contains a great number of organic admixtures, which are primarily of plant origin. Their biological action, which was first discovered by B. P. Tokin in the form of the bactericide effect, is extremely varied [87]. These substances include both inhibitors and stimulators of physiological functions, some of which have a vitamin-like action [90]. /2

Recently, similar concepts have been developed with regard to the microbe environment of humans, as the source of immunogenetic stimulation, which is a necessary condition for retaining the normal immunity of the organism [50].

Thus, the concept of the biological value and the adequacy of a biogenic living environment is not so much an assumption, as it is the result of the fact of the adaptive evolution on Earth. This fact is supported by diverse data from different branches of biology and medicine.

It thus follows that the most valuable living environment for the human being (and any organism on Earth) is a biogenic environment, i.e., a medium produced by biological methods, /3 which to a certain extent reproduce the mechanisms for the formation of the biosphere on the Earth.

If these considerations are taken into account, then the prospects for biological life support systems must be evaluated, not so much from the aspect of their economical use, but from the fact that conditions may arise when the use of biological systems becomes unavoidable due to the necessity of a living environment which is similar to conditions on the Earth.

Thus, biological systems are not simply one of the possible methods of regenerating individual substances: oxygen, food, and water. At a certain level of the organization of the biological life support system, a method arises which is unique in that it cannot be reproduced and which qualitatively differs from non-biological systems. This is the method of producing a biogenic human living environment which is most similar to conditions on Earth, and consequently satisfies the biological requirements of man.

The greatest advantages of a biological system can be realized to the greatest extent when individual biological cycles are combined into a single functional system of higher order, which does not simply regenerate the components of the medium which are necessary for man, but also produces this medium, independently of the inadequacy of our concepts regarding all the components of the medium which are necessary for prolonged autonomous existence of man.

One of the main advantages of biological systems of any organizational level is the potential possibility of their stable functioning on the basis of internal control mechanisms /4 with a minimum (in comparison with non-biological systems) volume of the control and guidance function. This is related to the universal principle of the mutual correlation between processes in biological systems at all levels of the organization, beginning with the homeostatic self-control processes of metabolism and physiological functions in the cell, up to the internal mechanisms of stability maintenance at the level of population and biogeocenosis.

We shall investigate the biological methods of life support primarily in the aspect of a complete biological system with a relatively closed metabolic cycle. The construction of this type of system entails the greatest scientific-theoretical, experimental, and technical difficulties, but it is also of the greatest scientific interest. Based on an examination of the problem in this aspect, we shall be readily able to evaluate the possibilities for using individual biological sub-systems to solve the particular problems of biological regeneration of individual substances in mixed biological-physical or biological-chemical systems.

The development of a biological life support system lies in the sphere of theoretical and experimental biogeocenology. At the present time, it may be assumed that the development of such a system means including man in an artificially produced biocenosis, capable of relatively independent existence under conditions of prolonged dynamic equilibrium, primarily on the basis of internal control mechanisms in a relatively closed metabolic cycle.

For contemporary biology, this is a new problem, which does /5
not have adequate experimental and theoretical bases.

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The author would like to express his appreciation to them.
Before presenting this material, we would like to give certain of the most important concepts and terms pertaining to natural and experimental biological systems.

Basic Concepts and Terms /6

1. Abiotic medium — physical factors in the living environment of an organism.

2. Autotrophic organisms — organisms capable of synthesizing the organic compounds which they contain from inorganic compounds (carbon dioxide, water, mineral salts). Based on the source of energy used for organic synthesis, they may be divided into phototrophic, which use the electromagnetic emission in the visible spectral region, and chemotrophic, which receive energy as a result of their oxidation of different substances (iron, sulfur, hydrogen, nitrates).

3. Biotic medium — the entire living environment of any organism.

4. Biomass — the sum of all substances comprising the organism.

5. Biosphere — the current concept is based on V. I. Vernadskiy [14], and designates a combination of mixed portions of the lithosphere (solid covering of the Earth), hydrosphere, and atmosphere, which are connected (now or historically) with the activity of the organisms populating them.

6. Biocenosis — functionally related set (group) of organisms which occupy a land or water region which is common to all of them. Biocenoses of plants (phytocenoses), animals (zoocenoses), and microorganisms (micro-biocenoses) may be individually distinguished.

7. Biogeocenosis — the group of a certain biocenosis with a complex of factors in the abiotic medium. According to N. V. Timofeyev-Resovskiy [86,b], biogeocenosis represents an elementary structural and functional subdivision of the biosphere, which performs a certain type of biogeochemical work /7 — metabolism. The author has established biogeocenosis as part of the biosphere, through which not one biocenotic, soil, geomorphological, or climatic boundary passes.

8. Heterotrophic organisms are organisms which are not capable of primary organic synthesis, and require prepared organic substances for their existence.

9. Biological life support system (BLSS) — a set of organisms, artificially produced in an organic region (water region) and abiotic factors, of the biogeocenosis type. In the functional sense, BLSS is a functionally unique animal-vegetable-microbe group, including man, which exists in the state of dynamic equilibrium on the basis of a relatively closed metabolic cycle.

We shall call individual functional links of a biological system relatively isolated populations or groups of autotrophic or heterotrophic macro- or micro-organisms, which perform a certain stage of the system metabolic cycle. The heterotrophic link will include the crew of a spacecraft, who perform their work based on the consumption of oxygen liberated by plants and the utilization of organic material produced in the system, with its decomposition into water, carbon dioxide, and mineral salts.

10. Population — one type of species which populate a relatively isolated homogeneous land region (water region).

11. Ecological food — a position occupied by a certain /8
form of biocenosis in metabolism, as well as its biogeochemical function in biogeocenosis. It may be realized in a group of material-energy relations between species of this type with their biotic and abiotic mediums. In contrast to the biotopic concept, ecological food is a functional concept, and not a landscape-territorial concept.

12. Ecological system — in contrast to biogeocenosis, as an elementary structural and functional unit of the biosphere, it is applied to biological systems of any scale, from the ocean to individual pools or stumps of trees. Thus, the lack of a size criterion makes it possible to apply this term more broadly than biogeocenosis.

However, it nevertheless applies to biological systems, and in the USSR literature it is not used for the identification of inhabited structures with non-biological systems providing for their habitation — for example, with physical-chemical systems.

13. Closed ecological system — a hypothetical biological system existing on the basis of a closed metabolic cycle without an exchange of matter over its boundary.

I. Certain Properties of Natural Biological Systems and a Modeled Biological Life Support System /9

The energy basis of biological metabolism is the assimilation of energy from outer sources, its transformation and accumulation in the organism in the form of energy-producing organic compounds. One source of energy may be the oxidation of certain inorganic compounds or elements (chemical-synthesizing bacteria), but the source most widely found in nature is the use of energy in the visible portion of solar radiation (green plants and certain bacteria).

This process takes place in the organism of plants during photosynthesis, and may be represented as follows:

$$CO_2 + H_2O + \left| \frac{\text{light}}{\text{chlorophyll}} \quad (CH_2O) + O_2 - 112 \right| \text{ kcal}$$

The energy of light is used in this process for the decomposition of water, which is connected in a very complex — and still unclarified — way with the synthesis of macroergic phosphate compounds, which serve as a reservoir of energy for the subsequent stage of synthesis of hydrocarbons, protein, lipides, nucleic

acids, and the materials of secondary metabolism in the plant organism. As may be seen, the basis of the biological regeneration of air, and the reproduction of food, lies in the photosynthesis reaction, since it is accompanied by the absorption of carbon dioxide, the liberation of free oxygen, and the accumulation of organic matter.

In the respiration process of heterotrophic organisms, oxidation of organic matter occurs with the liberation of energy accumulated in them and their conversion into carbon dioxide, water, and mineral materials. Their repeated conversion into /10
organic compounds which have a higher level of potential energy requires their consumption from external sources, which again occurs in green plants.

Thus, two opposed processes lie at the basis of metabolism in biological systems: primary (autotrophic) synthesis of organic substances with the accumulation in them of free energy, and the destruction of these substances with the mobilization of accumulated energy which is used in the vital activitiy of the organisms.

In accordance with this, a scheme for closed metabolism consists of two semi-cycles in principle — synthesis and destruction — which may be represented by the organisms with autotrophic and heterotrophic types of metabolism. In natural biocenoses, which represent complex systems of several species with a large number of parallel and sequential links, the real material-energy relationships are also imposed on this scheme in principle, although specific relations may not always be apparent due to the great complexity of natural systems.

Figure 1 shows diagrams of relationships in a multi-stage biological system, comprising a sequential trophic circuit. The first trophic level is composed of photosynthesizing plants, and the second — herbivores. The latter may serve as food for the carnivores (beasts of prey of the first order), which in their turn may occur in the food system of beasts of prey of the third order, etc. Each trophic level is usually occupied by several competing species.

In the final analysis, the last stage of the food cycle /11
is different heterotrophic micro-organisms, which complete the process of destruction of organic material and its conversion into inorganic compounds, which are again used by the plants in the subsequent cycle of synthesis of organic substances and accumulation of energy. The conversion of energy from one trophic level to another is completed with a loss of a significant (more than 90%) amount of energy. In this way, the total reserve of energy in each consecutive trophic level is decreased as compared with the previous one. For artificial biological systems of life support, to maintain the vital activity of a certain mass of organisms at a given trophic level, the production of a much greater mass of organisms at the subsequent levels is necessary. Therefore, an increase in the number of trophic levels which provide the human food cycle in BLSS becomes disadvantageous.

A unique example of such a system is nature on the Earth — more precisely, that portion of it which was distinguished and regarded by V. I. Vernadskiy [14] as the biosphere — one of the outer envelopes of our planet occupied by living organisms and inorganic bodies which have a direct material-energy exchange with living organisms. A characteristic feature of the biosphere is the prolonged state of dynamic equilibrium between living components of the abiotic medium.

Usually, the extent to which biological metabolism is closed is not great in nature, keeping in mind the outflow of a large amount of biogenic carbon from the cycle and its accumulation in combustible minerals, limestones, and other carbonate rocks. Actually, the amount of organic carbon, which is bound /12 in limestones and coals, may be established as three orders of magnitude greater than the amount of carbon in living matter on the planet. However, if the fact is taken into account that life on the Earth and, consequently, the biosphere has existed for about 2.5 — 3 billion years, it is found that the yearly outflow of carbon from the biological cycle during this time has been about $1 \cdot 10^7$ tons per year on the average, i.e., on the order of thousandths of a percent of the yearly production of plant biomass of the planet, which is estimated according to recent data as $2.3 \cdot 10^{11}$ tons of dry material per year [8].

There is no doubt that such a high degree of closure follows directly from the enormous functional diversity of existing cycles of organisms, which — with the very narrow individual specialization of their biochemical functions — are in a position to encompass all forms of organic matter and all products of its conversions with their transforming action.

It is understandable that such a degree of closure of the cycle of matter in artificially created biosystems is impractical — in fact it is not even necessary — since the time for the stable operation of an artificial system must not be measured in geological scales, but in a few years or tens of years.

Keeping in mind the practical problems, it is of interest to examine the extent to which existing data and certain characteristics of the cycle of matter in nature make it possible to do this, and to distinguish certain arbitrary values of them

as criteria for a comparison with proposed artificial systems. /13
Such criteria may be masses of animate and inanimate matter, their relationship, and also the circulation rate of the basic materials. These criteria may probably be included in a number of basic biotechnological characteristics of biological life support systems with unusual "similarity criteria" in a comparative evaluation of them.

Before presenting the existing data, we must stipulate the following: When establishing the absolute and specific (per unit of living matter) amount of the basic materials in the biosphere (water, oxygen, carbon dioxide) during the cycle, the fact must be taken into account that not all of the space in the hydrosphere and atmosphere is the location of very active processes of material exchange between the organisms and the medium. Thus, the most active (with respect to living processes) zone of the ocean is several tens of meters wide, which is determined by the total absorption of light at this depth and the impossibility of the basic process — photosynthesis.

In the atmosphere, we may also distinguish the lower layers of the troposphere, which most actively participate in the biological cycle, with the most clearly expressed processes of turbulent mixing of the air. Air layers near the Earth, which extend to certain phytocenoses at an altitude from several meters to several tens of meters (the woods of the humid tropical and subtropical zone) participate most actively in photosynthetic gas exchange. Since the basic source of carbon dioxide in the atmosphere is soil processes, in dense grasses and dense multi-layer tropical forests, the vegetation covering of dry land located along the path over which carbon dioxide is liberated and propagated is a powerful absorber for it. As a /14

result, respiratory and photosynthetic gas exchange is practically localized in a small layer of air near the Earth, where the rate of gas exchange is at a maximum, and the rate of wind agitation is at a minimum.

Table 1 presents the basic parameters of the biological cycle of matter and of an abiotic medium in the biosphere of the earth. On the basis of the statements just made, they are clearly too low with respect to the estimate of the amount of water and gases in the atmosphere which interact with living matter and which comprise the real space of terrestrial and aqueous photosynthesizing systems. Figure 2 will give the corresponding corrections on the basis of the considerations made above.

It may be seen from Table 1 that in the biosphere of the Earth for each unit of biomass there is (by weight) three orders of magnitude more air and five orders of magnitude more water. This is the power of the natural dampers which undoubtedly play an important role in providing stability for a system by smoothing out the fluctuations in the composition of the air and water medium. If we take into account only the zones of the greatest photosynthesis activity, as was done above, then the portion of water per unit of biomass decreases by two orders of magnitude, and the atmosphere — by at least one order of magnitude. Only in those zones such as the humid tropical forests does the effective volume of the atmosphere, which is practically excluded from the wind mixing with the upper atmospheric layers, decrease to the same order of magnitude as the amount of biomass (by weight) or to several m^3 per 1 kilogram of phytomass. This value /15 is close to the corresponding characteristics of existing hothouses and hotbeds.

An important functional index of the biosphere activity is the metabolic rate, which we may calculate from the data in Table 1. This is the ratio of the yearly increase or consumption of matter and its present reserve. Thus, for the biosphere as a whole, the rate at which the phytomass is formed is about 10 years, and consequently the metabolic rate of organic material is 0.1 circulations per year. However, this value is compiled as the average of very diverse restoration rates — for example, of forests on dry land — and of its basic phytomass, with a restoration period of its mass and the phytomass of the ocean extending over several years, where the rate of the organic material cycle is about 350 circulations per year (the yearly production of phytomass in the ocean is 350 times greater than its available amount).

Thus, Table 1 gives an idea about the real relationship of the basic components of the biosphere and the scales of the cycle of matter. It may be seen from the table that the amount of phytomass and carbon dioxide in the atmosphere is approximately equal by weight, and the rate of their restoration is about ten years. For the available reserve of atmospheric oxygen, this period is a thousand years, and for water in the ocean, it is on the order of a million years. It is apparent that the limiting link of the cycle in the biosphere is the cycle of carbon dioxide and the reproduction (which it limits) of a primary product. The stability of the cycle in the biosphere is determined by the stability of the reproductive cycle of phytomass and carbon dioxide, since the specific amounts of oxygen in the atmosphere and water in the ocean are much greater (by 2 — 5 orders of magnitude) than that of carbon dioxide. Therefore, the possible amplitudes of fluctuations in /16 their amounts are smaller, and the change to an unstable state is less probable.

It may be seen from Table 2 that in zones with the greatest concentration of life the specific amounts of inanimate matter and the rate at which it is restored is 1 — 2 orders of magnitude less than in the biosphere as a whole, although the production of these regions is only about 35% of the production of biomass in the entire biosphere.

Table 3 shows the possible scales of an artificial biological system, including a five-man crew. As may be seen, the ratio between the amount of carbon dioxide and biomass corresponds to the real, highly productive natural macro-biocenoses (see Table 2), and the specific amounts of the atmosphere and of oxygen per unit of animate matter is only one order of magnitude less than in a real natural macrosystem, like the rate at which the available amount of carbon dioxide is restored. Only the absolute dimensions of the system (the amount of animate matter and of atmospheric gases) are 13 — 15 orders of magnitude less than the dimensions of a natural system, which provides an idea of the enormous reduction in the scales of a model, as compared with nature. With such a change in the scales, a loss of the basic quality of the original — and the stability of its operation — is naturally unavoidable, since — within the framework of such a reduction in the amount of animate matter in the artifical system — it is impossible to retain all of the qualitative diversity of the animate components of the system, in which the activity of the organisms would completely encompass all forms of matter circulating in the model, i.e., a completely closed cycle of matter within it. On the other hand, we have already stipulated that this is not an absolute necessity for us.

Thus, it follows from an analysis of biological systems (biosphere and macrobiogeocenoses) that the basic factors for retaining a high degree of stability in the biological cycle of matter are a large amount of biogenic substances circulating outside of the organisms (which provides reliable damping of temporal and local functuations in the medium parameters) and a large amount of animate material when there is an enormous diversity of biogeochemical functions of different types of organisms (which provides a high degree of closure for the cycle of matter). Other basic factors are also repeated duplications of homogeneous functional elements (species of one type) and the presence of many parallel functional links in the form of different types of organisms occupying the overall ecological cycle. /17

Certain problems of adequately modeling biological systems, which are related to unequal scales in the model and the original, and problems of the critical mass of animate and inert matter in a system are examined in [95].

II. Line Diagram and Functional Structure of a Biological Life Support System /18

In this section, we shall examine only the most general problems of the BLSS structure, the purpose and function of the main elements, their relationship in the system, the possible solutions, and the principles underlying the selection of the forms of organisms, without examining the specific problems of biotechnology, which shall be the subject of a following chapter.

In accordance with the functional structure of natural systems, an artificial biological system must consist of the following main functional elements, which are shown in the block diagram (Figure 2).

Energy is introduced into the system by means of the photoautotrophic element, which may consist of different representatives of higher plants and unicellular algae.

Undoubtedly, the higher forms of plants, which are traditionally necessary for a food supply, must be the basic source of the food products of vegetative origin, in spite of such drawbacks as complicated cultivation under artificial conditions, inadequacy of the biomass obtained for supplying food for human beings, the frequent necessity for periodic illumination in the majority of cases, and the frequent lack of self-pollination ability.

The problem of grading the higher and lower forms of plants in a photoautotrophic element is examined in [66, 98] from the point of view of stability and control of the element.

From this point of view, the greatest drawback of a photoautotrophic element, which is based on the higher forms of plants, is its great inertia as a control object, particularly under /19
extreme conditions, which is related to the long cycle of individual development and periodic illumination. This causes long periods of restoration (many weeks) of the initial functional level after reversible damage under extreme conditions. Including unicellular algae in a photoautotrophic element significantly optimizes its functional characteristics, both along the line of the biochemical composition of the biomass produced (high content of protein and fat), as well as along the line of dynamic properties of the system. This is because unicellular algae, due to their high multiplication rate and the great ease with which they can be cultivated, are the least inert elements in a system for controlling autotrophic organisms. These characteristics of unicellular algae not only justify their inclusion

in a biological system, but also determine their minimal contribution to the composition of an autotrophic element.

If a certain variable component, dictated by the required control range, is included in the total gas exchange of a photoautotrophic element, this portion of the total productivity of the element will correspond to the minimum portion of the productivity of unicellular algae. Their upper limit in the autotrophic element will be determined by the maximum possibility of using them in the food supply.

The problem of the nutritive value of algae cannot yet be considered as solved, in spite of the significant amount of research already performed, especially in Japan, United States, and the Soviet Union.

The first experiments on animals provided positive results. Algae are quite rich in vitamins and protein. For example, /20
chl. pyunoidosa contains from 40 to 60% protein, about 20% carbohydrates, from 10 to 20% fat and 5 — 10% ash compounds. The amino acid composition of proteins in chlorella is completely adequate for the requirements of humans, with the exception of sulfur-containing amino acids — methionine and cystine — which are deficient in the biomass of algae. However, based on data compiled up to the present time, their permissible portion in the food supply of human beings does not exceed 20%, although in individual cases, the requirement for algae has reached 500 grams/day. One of the very reliable studies in this field is given in [128].

Our own experiments on including in a human food supply 50, 100, and 150 grams of a biomass mixture of chlorella and scenedesmus algae, cultivated in open greenhouses with natural illumination, showed that including 50 grams of algae (dry weight) in the food supply of three test subjects was accompanied by the development of dyspeptic phenomena (belching, nausea, and loss of appetite), which disappeared after 2 — 3 days [45].

In another group of test subjects, a food supply with 100 grams of dry mass of algae produced dyspeptic phenomena in the first few days in all four test subjects of this group. The period of subjective adaptation to this diet was more prolonged; however, no objective disturbances in the function of the gastroenteritic tract were observed throughout the entire experiment (20 days).

Out of five test subjects who received 150 grams of algae biomass per day, four displayed prolonged dyspeptic phenomena, as well as allergic reactions in the form of local hyperemia and edema on the face and distal portions of the extremities. In two cases, these phenomena appeared only after changing to a control diet.

In evaluating these results, the fact must be taken into account that the first studies on the nutritive value of algae biomass, including these experiments, were performed with a biomass of different species and strains, cultivated in containers under the open sky, with a large portion of uncontrolled microflora in the biomass. /21

Therefore, the reason for the allergic phenomena which we observed after unicellular algae were consumed in the food may be related both to the properties of the algae themselves, and to the bacterial admixtures in the non-sterile culture, particularly with biologically active lipopolysaccharide complexes of bacterial structures.

A special study of this problem revealed the absence of allergen properties of chlorella with the intracutaneous and subcutaneous introduction of preparations of their biomass in animals and humans [75].

Data which have been obtained up to the present indicate that the human food supply may contain, according to data of different authors, from 30 to 100 grams of dry algae mass per day. Reduced data on this problem are given in the summary article [10].

One of the disadvantages in using unicellular algae for food is their poor assimilability which is limited by the poorly digestible wall [116]. This led to the necessity of studying the possibility of enzymatic destruction of cell walls by means of the cellulose enzyme of certain bacteria, lower fungi and mollusks [117]. A positive result was obtained with the use of the enzyme obtained from the trichoderma bacteria [135]. The destruction of the cell walls with a subsequent increase in the degree to which algae protein could be digested was found in a /22 study on the effectiveness of cellulose derived from the alimentary canal of the Helix pomatia snail [11].

Positive results have also been obtained on the use of different methods for a physical destruction of the algae walls. However, these have required quite complex equipment and have encountered the problem of increasing the technical capacities of its operation [89].

Just as for higher forms of plants, the use of [unicellular] algae for food depends to a great extent on the variety of the biochemical composition of their biomass. At the present time, the use of [unicellular] algae as applied to life support systems has been limited to several representatives of closely related species of Protococcacaea, which do not have any essential differences in their biological composition or nutrient properties.

In the creation of an artificial biocenosis, it is important to establish the criteria for selecting the forms of plants and animals which are most advantageous for use in the system. Some of these criteria were examined in the studies [30, 127] with respect to selecting plants with the following qualities: high productivity, biochemical composition of the biomass satisfying the human requirements, customary use for human food, similar requirements on the external environment, compatibility between the plants themselves and humans, and the degree of technical complexity in preparing foods from the crop obtained. An example of complex technology is a system for obtaining bread from grains and cereals requiring five technological operations. In contrast to this is the simplicity with which salad greens may be used. /23

It must be stated that the problem of criteria to be used in selecting species for a biological system is quite complex and contradictory, which is explained by the contradictory requirements on any elementary life support system, when the system is used under the spaceflight conditions. These requirements for biological objects are primarily determined by two factors: the requirements of the human being, as the guiding link in the system, and the attempt to increase the effectiveness both of the biological system as a whole, and of each functional element (phyto- or zoocenosis). The first requirement is very frequently in contradiction to the remaining ones, and therefore the final selection

of biological objects will most frequently be ambiguous and will represent a compromise. The use of a compromise is apparently universal for the problem of life support, which is characterized by limitations in terms of weight, volume, and energy requirements.

Thus, for example, the requirement for an adequate composition of the vegetative portion of the human food supply encounters the necessity of including in the autotrophic element, for example, bean, grain, and oil cultures to provide the protein-fat portion of the diet. Based on these considerations, peanuts, beans, and lima beans were studied [119] for use in biological systems, and wheat was a component of the autotrophic element in an experiment with human beings [57, 58].

However, these cultures are least suitable in terms of the overall effectiveness of the element and of the system as a whole, since a comparatively small portion — seeds (grain, beans) — is used from the biomass produced in the human diet. The remaining portion of the biomass produced requires an unproductive outlay on its mineralization in order to be included in the subsequent cycle. The energy efficiency is thus sharply reduced, which without this is small in an autotrophic element. /24

Thus, the criteria of maximum "complete consumption" (complete use of the biomass produced in the system), which is necessary in terms of the effectiveness of the system, contradicts the requirements for the guiding element — man. In this case, a compromise makes it necessary to examine the possibility of minimizing the grain and oil plants in satisfying the nutritive requirements of man.

However, the importance of individual criteria may change, depending on the biocenotic structure of the system and the degree to which the cycle of matter is closed in this system. Thus, the introduction into the system of heterotrophic organisms, which require plants which are inedible for humans, increases the degree to which a system is closed and decreases the value of the above criterion of "complete consumption." The problem of the effectiveness of introducing a heterotrophic element into a system was partially examined in [25].

In spite of the contradiction between different criteria, we can point out the most general criteria which determine whether some forms of organisms are preferred over others. This is first of all the nutritive value for human beings, a high level of productivity (per unit of soil area, volume, or illuminated surface per unit of time) with the most complete use of the biomass produced in the system, commonality between the medium physical conditions in a group of several species, and biological compatibility in the case of concurrent cultivation (the absence of harmful mutual influences or direct competition), which simplifies the technique for cultivating the organisms in a limited area. /25

The combination of criteria of high specific productivity and common characteristics of the medium conditions leads us to search for preferred forms of plants among tropical and subtropical forms. This selection is also of value in terms of the optimum use of light in photoperiodic plants when there is an equal or similar duration of the light and dark period in tropical and subtropical forms.

However, with any criteria, the final selection of biological objects will always be a compromise, and is determined in the last analysis by the optimality of the system as a whole.

A closed cycle of food regeneration necessitates the introduction into the system of an element of heterotrophic synthesis, in order to obtain the necessary minimum of animal products, although this may decrease the overall efficiency of the system. Nevertheless, if we agree with the commonly accepted opinion that products of animal origin are necessary in the food supply, then the introduction of animals will be effective in any system where there are plant waste products which are edible for them, but are inedible for man.

Up to the present, calculations have been made of the material-energy balance of animals when rabbits and poultry are used for this purpose [1, 7]. For this purpose, preliminary studies were made of the possible use of certain tropical fish, for example, Tilapia mossambica [67, 68].

It is of interest to use the so-called "alienated" production of animals, which removes the problem of their propagation under space flight conditions. For this purpose, it has been proposed that goats, for example, be used to obtain milk. /26

The first specific proposals with respect to the forms of animals used pertain to certain plankton Crustacea — artemisia (Artemia salina) and Daphne (Daphnia magna) [106, 132].

Recently, several researchers have been interested in mollusks which can be fed by plant waste products and do not have shells (species Lymax, Arion, etc.).

A great number of contradictory selection criteria must be included when formulating the animal link. The most important criteria are suitability as food, high specific productivity, and continuous technical cultivation under artificial conditions.

Just as in any other biological link, general preference must be given to the small animals with the shortest cycle of individual development. This is because the accidental death of any individual disturbs the equilibrium of the living system; this disturbance is greater, the greater is the contribution of the disabled element to the total mass of the system or the given link. To increase the reliability of a system, it is advisable to select components with the smallest mass of the species and with the shortest cycle of individual development, which increases the ability of the element and the system as a whole to be restored in the case of possible disturbances of equilibrium. The high specific metabolic rate in the smaller organisms is also of value [15]. /27

The problem of the psychological acceptability of unusual sources of animal products is naturally of great value. However, it is impossible to examine this in a manner which is too straightforward, without taking into account the amount of unusual product required and particularly without taking into account the use of culinary technology, whose possibilities for changing the initial properties of the nutrient medium until it cannot be recognized are well known.

Man and animals in such a system can destroy (mineralize) up to 80 — 90% of the necessary organic material. However, in order to complete the mineralization of the products of man, animals, and plants, it is necessary to introduce a special mineralization link. The purpose of this is to mineralize the

organic material produced in the system and to create the conditions for the most complete closure of the metabolic cycle in the system.

We should recall that closure of the cycle is an area in which the self-control properties of the system may be realized. Therefore, the role of complete mineralization and utilization of organic waste products is particularly important to establish the maximum effectiveness of the BLSS. The completeness of the closure of the cycle of all biogenic elements and, consequently, the closure of gas exchange and water balance in the system depend on the extent to which organic waste products are utilized.

At the present time, two directions in the mineralization processes of organic waste products are being examined and studied: physical-chemical and biological. We previously discussed the preference of a biological mineralization method for biological systems in connection with a discussion of the biological value of a plant medium [95]. It may be assumed that the requirements of plants over several cycles of vegetation exceed those which may be represented by laboratory nutritive means. These do not take into account the important role played by the rhizosphere microflora in the life of plants, just like in general the role of the interaction of plants with soil microflora. These ideas enable us to understand the cessation of flowering of hydroponic cultures after several generations, which was discussed in the article by Noswald [126]. /28

Out of the microbiological mineralization processes, the anaerobic processes were rejected in the first examination due to their duration and the formation of incomplete oxidation products, including gaseous products (hydrogen, methane, etc.) which were not utilized directly in the biological system.

The biological method of mineralization of organic waste products may be based on processes of aerobic oxidation in "biological ponds," in microbiological reactors based on the type of air tank used to purify waste waters. Similar processes and devices were first used to analyze mixtures of urine and feces in algae reactors: Lynch [120], Golucke [112], and Tischer [113, 134], and for the mineralization of feces: Bovee [104], as well as a mixture of nutritive waste products and human vital products (David [108]).

Lanerg and Tischer [118] published the first summary of the data in the literature on this problem. Later, these processes were carefully studied with respect to the direct utilization of aerobic decomposition of feces and urine in the "human-algae" system, and also with respect to using urine which was first mineralized [53] and urine which was dried lyophilically [64] directly in an algae reactor. Meleshko and others showed that the direct use of urine in an algae reactor when the nutritive medium was recirculated is only possible for a limited period of time, which is limited by the period in which an excess amount (approximately 6 grams/liter) of chlorine ions are accumulated, which are not used by the algae [64]. A similar limitation with respect to creatinine and hippuric acid was shown by Lynch [120]. /29

These experiments provide an example of the accelerated experimental solution of the problem of macrobiocenoses in aqueous ecosystems used for purifying waste water. The salinization of many such tanks is an accomplished fact at the present time. We might point out the positive role played by model closed biosystems as highly sensitive indicators for predicting behavior of macrobiosystems under similar conditions.

All of these attempts to utilize urine in algae reactors point to the necessity of organizing a short-closed "human-human" cycle with respect to sodium chloride, which may be used by man to a significant extent as a palatable substance, and which may not be required in an equivalent amount by other components of the biological system. The solution of this problem — which most probably lies within the realm of physical chemistry — limits the complete utilization of biogenic elements of urine in the system.

Significant difficulties are encountered for a biological /30
method of mineralizing the organic matter of feces and plant waste products, consisting primarily of cellular tissue, which is very slowly decomposed by microorganisms.

Among the physico-chemical mineralization methods, studies have been made of both direct (thermal) combustion as well as oxidation in the liquid phase at high pressures and temperatures (so-called moist combustion). Some results of these studies are given in the work by Bovee [104], Gol'dshvend, et al. [28], Shorin, et al. [99], and Yazdovskiy, et al. [100].

However, subsequent studies have shown that the apparent simplicity of thermal or liquid phase oxidation is accompanied by the complexity of using the end products of these processes, due to the toxicity of some of them in liquid phase oxidation. According to the data of Agre [2, 3] this makes the products of moist combustion of feces and urine unsuitable for use by higher forms of plants without additional processing.

In the case of thermal combustion of feces, which has been thoroughly studied from the thermophysical aspect by Shorin, et al. [99], a problem arises which is difficult to solve under conditions of a closed system. This problem is connected with the recovery during the cycle of poorly soluble metal oxides from the sol obtained, as well as nitrogen, phosphorus, and sulfur from the gaseous phase. This is the basic drawback of the physical-chemical (thermal) mineralization.

The problem of binding molecular nitrogen led to the study of nitrogen-fixing micro-organisms, particularly of synthesized algae, as a means for eliminating the impasse in the cycle of nitrogen. Thus, Kordyum [46], who studied several representa- /31
tives of blue-green algae, showed the possibility of using them to bind up to 2.0 grams nitrogen per 1 square meter of illuminated surface per day (with around-the-clock illumination). According to the data of Antonyan [6], the blue-green algae (Anabaena variablus) may bind from six to twenty percent of the atmospheric nitrogen in the cells.

If we are discussing the future prospects, then real variations of the mineralization element in BLSS will naturally be to combine biological or physical-chemical mineralization methods with combustion, in a portion of the latter, of cellular tissue, whose combustion products are carbon dioxide and water. The technique of "moist combustion," in which the process is carried out at a pressure of 150 — 250 atmospheres or more, is extremely inefficient as compared with direct combustion, particularly since the end products obtained cannot be utilized without subsequent processing. Thus, the processes of physical-chemical mineralization and, in particular, thermal oxidation must be a component part of the mineralization system of organic waste products in BLSS.

The difficulties of utilizing the end products of physical-chemical mineralization in a closed system compell us to turn again to the advantages of the biological method, whose end products correspond to the plant requirements best of all. In addition, it should be recalled that the activity of micro-organisms in a system of biological mineralization is accompanied by the formation in the plant environment of vitamins, hormones, and other biologically active materials, which are of great biological value for the plant growing medium, as compared with /32 liquid from an autoclave or a solution of furnace ashes.

III. Problems and Prospects for the Development of Individual Functional Links in Biological Life Support Systems (Biotechnical Aspects)

Due to different circumstances, at the present time different functional links in biological systems are at different stages of study. In this chapter, we shall present the basic facts obtained from studying each link, and also the problems and prospects with which this study is confronted.

In 1966 in a report to the Ames Research Center, a comparative evaluation was made of the extent to which individual links in a biological system could be developed. This is presented in Table 4. In this table, the numbers in parentheses designate the estimates which should now be substituted after six years of intensive research. As may be seen from the table, the relative position of the research subjects in this table has not changed in this period.

The development of any link in a biological system first of all means selecting the most suitable forms of organisms and species of the link, which will subsequently be the subject of biotechnological research, as the functional link in the material-energy relationships in the system of the metabolic cycle.

A. Autotrophic Link

As was already indicated on page 18, the optimum variations of a photoautotrophic link may be compiled by combining unicellular algae and higher forms of plants. /33 Attempts which are published in the literature to regard unicellular algae as a single partner of man — not only in terms of gas exchange, but also in terms of nutritive relationships — are now of only historical value, and will not be studied here. Naturally, this does not exclude the fact that it is useful and necessary to examine the problem of utilizing photosynthetic gas exchangers as independent objects, as Myers [125] wrote. In addition, we may state with confidence that any biological subsystem of the BLSS must pass, during the process of its formation and technological processing, through the stage of an independent subsystem, which is duplicated by the appropriate physico-chemical devices or reserves, during flight tests on board a spacecraft or at a lunar base.

Unicellular algae

The first attempts to study the possibility of using the unicellular algae for the vital processes of spacecraft crews were made in the studies of Gafford and Croft [110], Gaume [111], Bowman [105], Myers [124], and Tischer [133].

A reliable physiological-biochemical and biophysical basis for the effectiveness of photosynthetic gas exchangers based on unicellular algae was presented in the report by Myers [125] in 1964. A year later, Lockheed [114] presented preliminary evaluations of life support systems with algae (and also with hydrogenous bacteria and higher forms of plants). In 1966, Miller and Ward [122] published the first review of experimental data based on the use of unicellular algae in biological systems /34
based on an analysis of 173 sources in the literature. These data are still just as valuable today.

In the USSR, the study of unicellular algae for use in biological life support systems (LSS) was first done by Chesnokov, Pinevich, et al. [94], and Semenko, Vladimirova, and Nichiporovich [84]. The generalized results of the more recent studies have been presented by Shepelev and Meleshko [96].

The initial stage of the comparative evaluation and selection of the most promising forms was already pre-established, since several similar forms of Protococcaceae algae, particularly from the chlorella species, served long ago as traditional objects for plant physiologists when studying the processes of photosynthesis. They have been studied so well, that it was simply assumed that they would be used as the potential component of biological systems.

Subsequently, as a result of selection thermophilic strains of chlorella were produced: TX-71105 [129], used in the United States, and Chl. pyrenoidosa Sp. K u Chl. vulgaris studied in the USSR.

Productivity of cultures

In earlier experiments performed on cultures of a small density (hundreds of thousands of cells per ml of suspension), up to 200 — 500 or more liters of culture were required for the gas exchange of one human [32, 114]. Therefore, to decrease the amount of algae suspension in the total weight of the photosynthetic gas exchanger, it was necessary to greatly increase the specific productivity of the suspension, i.e., the productivity per unit of volume. This led to the necessity of using culture densities which had not been studied previously. In turn, this made it necessary to examine the optimum level of such cultivation parameters as illumination, carbon dioxide concentration in the air [60], and mineral salts in the culture medium. /35

The large gradients of carbon dioxide concentration in suspensions of great density moved the problem of increasing the partial CO_2 pressure to the boundary of the gas-liquid field.

This automatically led to the necessity of creating carbon dioxide concentrators or of increasing the surface of the suspension in contact with the regenerated air, if we abandon the total CO_2 concentration for man and algae (not more than 7 mm Hg). Miller and Ward [122] indicated this in their article.

The same considerations pertain to the partial pressure of oxygen in a suspension, which will increase greatly in well illuminated, dense suspensions. The oppressive action of oxygen on autosynthesis has been well known since the time of Barburg. Figure 3 gives an example of this influence, which is borrowed from the work of Meleshko et al. [66,a].

All of this indicates that the continuous intense cultivation of algae cannot be directly based on existing physiological data on the optimum medium conditions, which have been obtained in experiments on suspensions with a very low density.

The widest suspension density range (up to 16.6 billion cells per millimeter of suspension, or about 128 milligrams algae dry weight per liter) was studied by Meleshko [59]. The maximum productivity of this suspension in terms of oxygen per day was 246 ℓ of O_2 from 1 liter of suspension. In all, two liters are sufficient to provide gas exchange for one human being. However, the anticipated weight of photosynthetic equipment and their auxiliary devices is so large that it makes no sense to attempt to decrease greatly the suspension volume in the reactors. /36

These data have the value of a limiting case. The practical use of suspensions with this density, where the volumes of the cellular mass and the culture medium are similar to each other, is extremely difficult, and raises new biotechnological problems. It is not advantageous to solve these problems, due to the large volume of the suspension.

At the present time, in reactors which actually provide human gas exchange 15 — 40 liters of suspension are used with a density of 7 — 15 grams/liter. In this case, we are talking about laboratory reactors, whose purpose is not to minimize the technical characteristics. With improved light distribution, this suspension volume can be greatly reduced.

It must be noted that, as a result of the complexity and contradictory nature of requirements in algae physiology, physiological optics and light technology, mass transfer, as well as due to existing weight and energy limitations on spacecraft

equipment, there are still no satisfactory examples of photosynthetic reactors, which could be used as prototypes of photosynthetic systems to be used on spacecraft.

The reactors used in different laboratories are based on different methods of illumination, mixing, and gas exchange of the suspension, and each laboratory has its own construction. For example, the results described earlier of studying dense cultures [59], were obtained from a method of cultivation in a thin film, similar to that used in reactors described by Fillips [109]. However, here the film was not produced as a result of centrifugal distribution of the suspension over a fixed illuminated surface, but was due to spreading it over the internal surface of a rotating cylinder, which was laid horizontally, in the absence of a dark region. /37

Reactors are used with internal and external sources of light, with direct illumination of the suspension and distribution of the light throughout the suspension by means of wedge-like light guides with different ratios between the illuminated and dark regions of the suspension, etc. In a collaborative situation, equipping several laboratories with a unified type of photosynthetic reactor would be of great advantage in future joint studies.

The next problem of a photosynthetic gas exchanger is a large output of culture fluid in the circulation cultivation method, retaining a given density. Only with a single doubling of the culture density per day does the daily amount of culture medium, separated from the reactor along with the crop, equal the suspension volume in the reactors, i.e., several tens of liters per person. This would require a great increase in the capacity of the equipment for regenerating and purifying the

medium from organic admixtures, from which, for example, only polysaccharides can be deposited in the medium by different forms of algae at a rate of 20 — 45 mg/l per day [114].

The solution of the problem was found in the method of continuous algae cultivation, by means of which it was possible to return the culture medium directly to the reactor, without its preliminary processing [62, 63]. The anticipated limitation of /38 this method, due to the accumulation of inhibiting concentrations of organic material, did not arise. This was due to the positive action of the heterotrophic microflora of the algae reactor.

In a non-sterile algae culture, an algae-bacteria group which is in relative equilibrium is produced. In this group, the activity of the heterotrophic microflora provides relative stabilization of the content of organic metabolites at a level which does not reduce the physiological activity of the algae [72, 73].

To stabilize the level of organic mixtures, a certain degree of flowability of the culture is important, since part of the culture medium together with the admixtures is carried into the centrifuged biomass. In addition, the accumulation of a certain amount of organic metabolism products in the medium stimulates the growth of algae, and is one of the factors providing the biological value of the medium.

Strictly speaking, we have not observed a final end to the increase in organic matter in the culture medium. After a few months or more and after the beginning of cultivation with recovery of the medium, there is an increase, although it is small, in the amount of organic matter. This points to the presence of certain substances in the culture medium which are

not decomposed by the micro-organisms. It is possible that, by analogy with natural processes taking place in tanks rich in organic matter and soil, there is an accumulation here of humus material which is difficult to decompose. It is possible that /39 in this case we are dealing with an impasse, which must be studied further.

The development of a method for the continuous culture of algae with recovery of the medium was impossible without developing a balanced nutritive medium, in which the content of mineral elements would fully coincide with their requirements by the cells. As is known, ordinary laboratory media do not satisfy these requirements.

In this connection, a study was made of the actual requirements of algae cells for mineral elements in a dense culture [22, 49, 40, 123, 124], as well as the removal from the reactor of mineral elements both with the biomass of the growing crop, and in the form of a residue of the culture medium which is lost with the centrifuged biomass of the cells [22, 52, 27, 49].

If it were sufficient to study the chemical composition of the biomass in order to produce a balanced nutritive medium, the stabilization of the medium mineral background, with the continuous harvesting of the crop, would require the creation of a special correcting medium, whose composition took into account all forms of the element loss from the reactor, including their sorbtion on the cell surface.

These data were obtained for different strains of chlorella and different nitrogen sources in a nutritive medium (see Table 5).

On the basis of the data obtained by Lebedev et al. [51], a formula was proposed for a corrective mixture on the basis of nitrogen nitrate added to the culture medium per each gram of biomass grown (in dry weight):

HNO_3	—	0.300	$MgSo_4FH_2O$	—	0.059
KH_2PO_4	—	0.057	H_2SO_4	—	0.010
H_3PO_4	—	0.011	$FeSO_4$	—	0.0015

/40

The subsequent quantitative study of combined processes of cell metabolism in the culture showed a rigorous relationship between the requirements of the cells for mineral elements, gas exchange, and accumulation of biomass of a specific composition. Thus, according to the data of Meleshko and his coworkers, with the formation of one gram dry weight of chlorella, one liter of CO_2 is absorbed, and 1.2 liters of oxygen is liberated [96], or 0.9 and 1.015 normal liters. Thus, biomass is accumulated which is characterized by the formula $C_{6.6}H_{12.1}O_{3.5}$, which is similar to the formula $C_{6.0}H_{11.1}O_{2.7}$ given in Myers [125].

The amount of carbon dioxide which is absorbed by one gram of cell growth falls satisfactorily within the range of the carbon content in the biomass, which comprises 500 mg/g [96] and 453 mg/g [22], based on different determinations.

This agreement between the metabolic parameters of algae was used to select the controlling parameter when stabilizing the composition of the nutritive medium. The principle underlying the correlation between physiological functions in the organism makes it possible to use for this purpose any parameter from among those which have a correlative relationship. In this case, the mineral supply is most advantageously controlled both in terms of biomass growth, and carbon dioxide requirements [61, 65].

When using algae to provide gas exchange for human beings, the important problem always remains of the lack of agreement between the photosynthetic coefficient for algae and the respiratory coefficient for humans (the volumetric relationship /41 $CO_2:O_2$). In a study of this problem, it was found that the photosynthetic coefficient for algae depends on the form of the nitrogen supply [61, 125]. When nitrogen nitrate is used, it is minimal and amounts to 0.79 ± 0.01. When nitrogen is in the form of ammonia or urea, it is 0.82 — 85 and 0.99, respectively. On the basis of this, Meleshko proposed dividing algae reactors into sections and utilizing different forms of nitrogen supply for the algae in different sections. In principle, this makes it possible to obtain the necessary photosynthetic coefficient between 0.79 — 0.99, which encompasses all possible fluctuations of the human respiratory coefficient.

Utilization of algae in food

The scales on which algae may be used in the human food supply are limited by, among other reasons, insufficient carbohydrates in their biomass and an excess of protein, along with an insufficient amount of sulfur-containing amino acids. This has produced a large number of studies aimed at finding the means and methods for having the algae biomass chemical composition approach the human food requirements. Naturally, we do not have in mind here an absolute approximation. There is no necessity of this, since neither chlorella, nor any other organism can be the sole source of food for human beings. However, removing the basic disproportions in the biochemical composition of algae could contribute to an increase in their proportion in the food supply.

There are three ways to approach the solution of this problem. One of them was given in the study of K. V. Kvitko and his coworkers. By the artificial production of mutations, and subsequent breeding of the mutants, they obtained strains of chlorella with a quantity of cysteine which was four times greater than its content in the initial form, with an insignificant reduction in the culture productivity [96]. /42

In later studies on the nutritive properties of the biomass of mutants in rats, it was found that the amount of biomass of the mutant strain which was eaten up in a mixed diet was 85 — 100% as compared to 40 — 57% in the initial strain. The assimilability of the mutant strain biomass was 70 — 90%, with 53 — 83% assimilability of the standard strain. (Verzilin, et al. [12]). However, in a dense culture, the competitiveness of the mutants was reduced, with respect to reversible forms of the initial type, which leads to the gradual elimination of the mutant strain during prolonged cultivation [37].

The second approach to this research is represented by several studies on the qualitative direction of the biosynthesis of micro-algae from the laboratories of V. Ye. Semenko (USSR Academy of Sciences, Institute of Plant Physiology) and I. I. Gitel'son (USSR Academy of Sciences, Institute of Physics). These studies are based on the fact that the chemical composition of the algae biomass changes under extreme cultivation conditions.

Thus, under conditions of nitrogen deficiency (up to the complete absence of nitrogen in the medium) in chlorella there is intensified synthesis of lipids with an increase in their content of free aliphatic acids up to 75%, and a decrease in their degree of saturation (Klyachko, Gurvich, and Semenko [44]). /43

Thus, the amount of oleic acid in the total acids increases from 3.9 to 43%. The carbohydrate content in Chl. Sp. K, in the absence of nitrogen in the medium, may amount to 55% of the dry weight of the biomass. It is thus important that up to 80% of the carbohydrates in a given strain are represented by starch. When studying a large number of algae strains, the authors showed that the ability of certain substances to undergo directional synthesis is determined genetically, and is manifested in different ways in different forms and strains of algae.

Thus, the results obtained when Chl. vulgaris was grown in a medium devoid of nitrogen were studied by Sadikova et al. [83]. Figure 4 gives the basic results, based on material from this study. It may be seen from the figure that the general direction taken by changes in the organic composition of the cell biomass is in general the same as in a previous study [44]. However, attention should be called to the fact that a considerable portion of the carbohydrate increase does not fall on starch, as in the Chlorella Sp. K. strain, but on hemicellulose. The amount of this inert (in the nutritive sense) substance after twelve hours of cultivation represented 25% of the entire biomass, and after 24 hours — 30%.

This change naturally has nothing in common with optimization of the chemical composition and the nutritive value of the algae biomass.

Figure 4 calls attention to the sharp drop in the algae productivity during the first hours of cultivation in a medium devoid of nitrogen, which corroborates the extreme nature of the action. The authors established the fact that a decrease in the algae productivity begins when there is a decrease in in the content of nitrogen in the biomass up to 75 mg/g dry

material and less. With a nitrogen content in the cells of less /44
than 50 mg/g, their growth stops.

Another manner in which the composition of the algae organic matter is changed is related to the influence of extreme temperatures upon them. Figure 5, which was borrowed from a study by Semenenko et al. [85] shows, that with an increase in the suspension temperature from 37 to 43° C, there is a sharp change to an increase of carbohydrates in the biomass, with a simultaneous reduction in the protein content. The lipid content in the biomass hardly changes. Table 6, which was also borrowed from the study of Semenenko et al., gives the limits of the changes produced under the action of extreme temperatures, as compared with the customary cultivation conditions and with the average composition of a human diet. From this comparison, it may be seen that the disproportion between the biomass composition and the human diet is smoothed out under the action of extreme temperatures on the algae. It is possible that this may point to the possibility of increasing the amount of algae in human trophic systems and other BLSS heterotrophic organisms.

However, to decisively solve this problem, further research is necessary, particularly with respect to the composition and nutritive value of synthesized lipids and carbohydrates. In any case, the reactions of micro-organisms to extreme (unfavorable) conditions in the medium are related not only to rearrangement of the protoplasm composition, but also to accumulation of wall material (encapsulation, encystment). Based on this, not all extreme influences, if we take all of these results into account, may lead to actual optimization of the biochemical composition /45
and an increase in the nutritive value of the algae biomass.

The third approach to optimizing the biochemical composition of the algae may be implemented on a biocenotic level, if no individual species or strain of algae is used as the optimization object, but rather a multi-species link of algae in the LSS. In this case, the problem of optimizing the composition of the biomass produced may be solved by attempting to combine different forms of algae, with a different direction of biosynthesis under conditions which are normal for them [66, 98]. From this point of view, it is not the nearest relatives of Chlorella which are of the greatest interest, but the representatives of far removed taxonomic and ecological groups of algae — for example, flagellates — which up to now have belonged to the Protozoa phylum. In particular, in the work of Gromov et al. [29], attention is called to the prospects for the algae Chlamidomonas and Chlorococcum.

With our coworkers, [98] we studied certain characteristics of the Chlamidomonas reinhardii algae, which are of interest for use in biological systems.

Morphologically, these are rather large, oval cells up to 20 microns long (we should note that the larger cells may possibly have a relatively small amount of wall material). The protoplasm of adult cells contains a large amount of inclusions (Figure 6), predominantly of a carbohydrate nature.

The productivity of these algae in a dense culture amounts to 65 — 70% of the productivity of Chlorella which has been well studied. Table 7 gives a comparative estimate of the composition of the organic material of certain forms of algae which represent rather far removed (in the systematic sense) groups of Protococcaceae, blue-green, and flagellate algae. On the basis of the data obtained, the authors assume that the clearly /46

expressed carbohydrate direction of normal biosynthesis of the form of Chlamydomonadina form studied — which has quite high productivity under rigorous conditions — fully justifies including this phylum, along with Chlorella, in the composition of the algae link of a biological life support system [98].

Productivity control

In order to organize general control of the culture productivity during the prolonged use of photosynthetic systems, it is necessary to know the dynamic characteristics of the algae culture. Meleshko [65] studied the natural productivity fluctuations in a dense culture during prolonged experiments under stable cultivation conditions. It was found that the algae productivity spontaneously changes according to a periodic law with an amplitude up to 25 — 30% of the average productivity during the period being studied. These productivity fluctuations may be of practical importance in the development of photosynthetic systems. Figure 7 gives an example of these fluctuations. It may be seen from the figure that the culture productivity has a clearly expressed periodic character with the fluctuation period of about 12 — 14 days. Fluctuations of a smaller frequency, with a period of about 3 — 4 days, may be seen on the background of slow fluctuations.

The nature of the transitional processes in the culture with a change in the cultivation conditions was first studied by Ivanov and Aleksandrov [4, 31] with a step-wise change in the illumination. The transitional period was oscillatory in nature. It lasted 8 — 12 minutes in a culture with low density and with the use of high-frequency and low-inertia methods of determining the photosynthesis rate (capillary-manometric and polarographic). In the case of abrupt changes in the suspension /47

temperature between 36 — 42° C, the duration of the transitional process was 25 — 30 minutes [5].

In cultures with a density an order of magnitude greater than 7 — 12 g/l, and when measuring the photosynthesis rate based on the change in the suspension density, the duration of the transitional process with a step-wise illumination change was much greater — from 3 to 160 minutes [4, 19]. In this case, the magnitude and form of the transitional process, just like the physiological characteristics of the cells, change greatly as a function of the specific technological conditions and the structural features of each reactor (suspension density, its mixing rate, the presence of unilluminated suspension volumes, etc.).

Toxic admixtures

In the first studies on algae, data on the liberation of carbon monoxide by cells were used as the basis for a photosynthetic-exchanger: Wilks [138], Gafford and Craft [110], Bates [102], Myers [124].

Koratayev et al. [47] showed that the specific accumulation of carbon monoxide in a regenerated atmosphere has an inverse relationship with the photosynthesis rate. This was later /48
confirmed on a great amount of experimental material by Kazakov et al. [33, 34, 35]. The authors showed that the carbon monoxide content in a closed air circuit of a photosynthetic reactor is stabilized after a certain period of time, and this occurs more rapidly, if the air circuit for the algae is closed by the cabin with humans.

At the same time, the algae culture can absorb certain admixtures from the air, including carbon monoxide [32,a, 48].

Apparently, relative equilibrium occurs in this system between the processes of liberation and absorption of CO, which take place in the opposite direction, and this stabilizes its concentration in the atmosphere.

General characteristics of unicellular algae, as a functional BLSS link

At the present time, the biotechnical basis for an algae gas exchanger is prolonged cultivation of algae in suspensions with high density, with direct recirculation of the nutritive medium. That portion of water which is drawn off from the reactor in the moist biomass may be returned, if necessary, to the reactor in the process of drying the biomass. If the condensation of water vapor from the air which has left the gas exchanger is added to this, the water circuit in it can be completely closed.

Existing data show that an algae reactor in a BLSS may perform several functions. The first function is to provide human gas exchange. When the algae are supplied with mineral salts, this function may be performed with an increase in the excess oxygen or carbon dioxide in the air, which requires the appropriate correction. The magnitude of this correction for one of the gases will be up to 5 — 7% of the value for human gas exchange, depending upon the difference between the photosynthetic coefficients for algae and the respiratory coefficient for humans. When using a sectioned reactor and different forms of the nitrogen supply in different sections, this difference may be decreased or eliminated. /49

The air leaving an algae reactor is saturated with water vapors. When urine or everyday water which is uncontaminated by cleansing agents are used in the reactor, the reactor fulfills the role of evaporator in the water regeneration system, and also performs the function of an ammonia absorber, which is well utilized by the algae as a source of nitrogen. Thus, the reactor performs part of the function of an air purification filter. The possibility is not excluded that further research will reveal the possible expansion of this function of a reactor.

Finally, at least a portion of the algae biomass, which is produced in the form of a harvest, may be used in the food.

In current evaluations of the prospects for unicellular algae in terms of the weight criteria, they are compared with physico-chemical gas exchangers. There would be more validity in evaluations which take into account the semi-functional nature of an algae culture in a BLSS. But we are still left with a criterion which has not been evaluated quantitatively; this criterion is related to the qualitative difference between a biogenic atmosphere and the chemical solution of oxygen in nitrogen.

In flights of long duration, this difference may be decisive for producing an air medium which is biologically acceptable (similar to terrestrial conditions). /50

Higher Forms of Plants

In terms of their participation in the overall metabolic cycle, higher forms of plants will probably comprise the basic portion of the photoautotrophic link of BLSS. However, their role as a functional link in the entire biological system has been studied much less than that of unicellular algae.

Among the first representatives of the higher (vascular) plants which were studied with respect to the creation of biological systems, plants were primitively developed from the Leminaceae family which did not have a developed stem, grow in water or on its surface, or multiply primarily by vegetative means.

Based on data from the Lockheed firm [114], the first studies of representatives of this group of plants from the Lemna, Volfia and Spirodela genus were performed by Wilkes in 1962 — 1964. They showed that the gas exchange for one human being by means of Leminaceae photosynthesis requires 75 — 100 liters of culture medium and 25 kW electric power. The rate at which Leminaceae grows was studied in a rotating tank with an illuminated surface of 1.16 square meters in the stored culture. The growth amounted to 3.3 grams of dry matter per day [114].

The biomass of one of the Leminaceae (Spirodela polyurhiza) consisted of 33% protein, 39% carbohydrates, 4.9% cellulose, 5.3% fat, and 13% ash. However, the dry material of /51
these plants comprised 4% in all of their biomass. The remaining amount was in the form of water. Therefore, the indices barely differ from many vegetable plants, and are greatly inferior to those of unicellular algae, whose dry material in the majority of cases amounts to 20 — 33% of the entire biomass [114].

Further research on higher forms of plants has been done on the use of cultured plants (vegetables, legumes, and grains).

With respect to the higher forms of plants, we still have no solutions for such problems (which are necessary for a closed system) as the actual requirements of plants for mineral elements with a given method and cultivation conditions, the gas exchange function of plants, and its quantitative correlation with the requirement for mineral elements and the synthesis of biomass with a specific composition. We are still at the stage of developing means and methods for stabilizing the conditions for supplying minerals to plants in a long culture in an unchanging nutritive medium.

We still have no sufficient bases for discussing any definite (in terms of species composition) variations of a single link of higher forms of plants as part of BLSS. There are separate suggestions regarding the use of several plants in biological systems, which most frequently include different types of cabbages, lettuce, beets, and also carrots, potatos, sweet potatoes, beans, kidney beans, wheat, rice, onions, and dill. Several starchy tropical plants are also recommended: Chinese yam, taro, cassava, Jerusalem artichoke (Earth group), and from the oil producing plants — peanuts [30, 55, 56, 57, 114, 119, 126].

There are a few assumptions regarding the species structure of planting for higher forms of plants which would approximate the structure of the human diet. Table 8 presents certain variations of plantings. The data in this table give an idea of the planting area proposed, which is necessary to provide the plant component of the human diet.

Cultivation methods

Two groups of methods for the artificial cultivation of plants have been studied with respect to biological systems: the substrate and non-substrate cultures. The method by which the nutrient solution is supplied to the plants determines the distinction between hydroponics and aeroponics.

Hydroponics supply the mineral requirements to the plants by periodically supplying the nutrient solution to the roots of plants located in any substrate, or with no substrate at all. Figure 8 shows a line drawing of the hydroponic substrate device. In the aeroponic method of cultivation, the nutritive solution is supplied to the plant roots which are freely arranged (without a substrate) in a very dispersed state, in the form of a "fog," by means of sprayers. The line drawing of an aeroponic device is shown in Figure 9.

The basic advantages of the aeroponic culture consist of the fact that with this method the minimum amount of nutritive solution may be obtained per unit of planting area or biomass of the /53 cultivated plants. This leads to a significant reduction in the weight of the cultivation devices, whose expediency may be decisively determined only in an analysis of other characteristics of the aeroponic method.

This advantage of the method is extremely important for studying the dynamics of the absorption by plants of mineral elements, since in this way the maximum sensitivity of the experimental system to the process being studied is achieved. However, aeroponics can hardly be recommended as a method for prolonged productive culture, at least not until the possibility of failure is eliminated in the operation of the sprayers due to their

obstruction by dense particles, which are unavoidably formed in the root zone of the plants.

The decrease in the relative volume of the nutritive solution in aeroponic devices has a negative value. In particular, it leads to a more rapid accumulation of toxic plant metabolism products when they are grown on an unchanging medium [92].

Just as with the non-substrate method, a specific problem which is intrinsic to it arises in aeroponics. The absence of any substrate for the plant root system eliminates its normal relationship with rhizospheric microflora, which are intrinsic to these plants under normal soil conditions.

As is known, in terms of its historical origin, the root medium is the result of the joint activity of plants and microorganisms in a specific abiotic medium. This leads to the conclusion that the medium properties, which are necessary for the plant in its root zone, cannot be adequately described simply by /54
formulas of the nutritive media, i.e., by the relationship and amount of nitrogen, potassium, phosphorus, and other mineral elements, without allowance for the biotic medium in the root zone.

It follows from this that the processes of microbiological decomposition of organic waste products, which we have formally related to the activity of an isolated mineralization link, must take place in a certain portion of it, which we have still not completely established, throughout the region of the plant root system, in direct contact with it and the rhizospheric microflora.

Apparently, there is still no basis for asserting that non-substrate plant cultivation methods are in a position to produce a biologically suitable medium in tens of generations of self-reproducing populations. There is information in the literature to the effect that even in a substrate hydroponic culture the flowering of plants stops after several vegetations [126].

It is possible that, to obtain a long plant culture in a biological system, it may be necessary to use artificial substrates, which could contribute to the creation of more suitable aerobiological conditions.

The successful completion of the "Apollo" program made it possible to study the first variation for the substrate having a space origin: "Studying samples of the Lunar soil, collected by the 'Apollo' crew on the Earth, Doctor of Botanical Sciences Ch. Uolkinshoy, coworker at the Manned Spaceflight Center (Houston, Texas) established that the lunar dust was suitable for cultivating agricultural plants." Dr. Uolkinshoy assumes that there is a growth stimulator in the lunar dust.* /55

A preliminary evaluation of certain ceramic materials on the Earth, based on their physical characteristics, revealed the applicability of some of them (vermiculite and cermet) as substrates for hydroponics [54]. These substrates were used in many later studies. One typical difficulty encountered in using the substrate cultures was not only the absence of chemical inertness in the majority of them, but also their high sorbtion properties, which contribute to the accumulation of a disbalance between the plant requirements for mineral elements and their

*According to data of the author of this chapter, Doctor of Medicine, U. Jones.

actual ratio in the nutritive medium. In this case, the problem arises of a balanced nutritive medium, which we have already examined with the example of unicellular algae.

Rusakov studied the agrochemical properties of cermet on the basis of its nine-month use in hydroponic devices [31].

An important feature of the continuous cultivation of plants under the conditions of a biological life support system is the "conveyor line" method, i.e., different planting periods, in which plants of all ages are grown simultaneously, and the operations of planting and harvesting may be performed simultaneously in different sections of the planting area in specific intervals of time, which are the "spacing" of the conveyors. /56

Initially, the method of a "plant conveyor line" arose in livestock breeding to obtain a continuous supply of vitamins from the sprouting seeds of grains.

A great amount of research has recently been devoted to the agrotechnical problems of cultivation primarily of vegetable plants using the non-substrate and substrate method of growing them, the determination of the optimum plant density, the method of preparing seedlings and placing the plants in non-substrate devices, and a determination of the optimum regime for periodically feeding the nutritive solution to the plants [55, 56, 57, 58]. Table 9 gives a formula of the solutions for hydroponics.

Primary data were obtained on the real amounts of crops for different plants in hydroponic and aeroponic devices. By way of an example, Table 10 also gives data obtained from a large amount of experiments in the Institute of Medical-Biological Problems of the MZ SSSR (Laboratory of Professor Dadykin). It

is interesting that the potato crop indicated in this table was obtained with a non-substrate method of cultivation, which apparently was the first case of this.

However, in spite of the great amount of studies on higher forms of life, a solution has still not been reached to the problem of the conditions and methods for cultivating plants on an unchanging (recirculating) nutritive medium, as was done for unicellular algae, and a study has not been made of the role of microflora of the nutritive solution and the plant root zone.

In terms of photosynthetic gas exchange, the principal /57
difference between the higher forms of plants and algae lies in their direct exchange of gases with the atmosphere, without an intermediate aqueous medium [114]. This advantage of higher forms of plants eliminates the problem of concentrating carbon dioxide in order to provide its necessary rate of exchange, as compared with the "gas-liquid-cell" system. The carbon dioxide concentration in the air which is permissible for humans (no more than 1% at normal atmospheric pressure) more than satisfies the requirements of higher forms of plants, in which the saturation of photosynthesis occurs for a carbon dioxide content in the air of no more than 0.3%.

From the viewpoint of a closed system, the study of the photosynthetic and respiratory gas exchange of plants, with a differing concentration of oxygen and carbon dioxide in the air, performed by Nilovskaya and Bokovaya [69, 70], is of great interest. It was shown in these studies that the well-known Warburg effect, showing that there is a reduction in the photosynthesis rate with an increased oxygen content in the atmosphere, may be of practical importance in the cultivation of plants with the purpose of regenerating the atmosphere.

The data in Table 11 show that this effect differs not only for different plants, but also for different illumination. An increase in the light flux intensity from 100 to 200 W/m^2 [illegible] decreases the depressive influence of increased oxygen concentrations upon photosynthesis by a factor of 1.5 — 2.

These authors showed that an increase in the oxygen content in the air from 20 — 22% to 24 — 26% almost doubles the amount of carbon dioxide liberated in the night time, and increases the /58
proportion of night time respiration from 4 to 9%, from the value of the apparent photosynthesis during the daytime.

Utilization of waste products

The problems of the utilization by higher forms of plants of the native or mineralized vital products of man have not been systematically studied sufficiently.

It is interesting to recall that this problem is connected with the name of one of the pioneers of cosmonautics and rocket technology — A. F. Tsander. Apparently, the first experimental studies in this region were his experiments on growing vegetables in an artificial substrate made of wood coal, using unprocessed vital human products to feed them [91].

Presently, there are only a few works on this problem, which represent preliminary studies on the presence or absence of toxicity in the mineralization products of organic waste products with different mineralization methods [3, 92, 100]. One of these studies points out the rather paradoxical phenomenon of an increase in the salt-tolerance of leafy cabbage when they were grown with the use of urine mineralization products [92]. It was shown in this study that the use of urine as a source of

nitrogen and other elements is limited by the accumulation of chlorine ions which are not used by the plants.

Thus, just as in the cultivation of algae, a problem is encountered when changing to cultivation from recirculation of the nutritive medium. For the higher forms of plants, a closed cycle of waste product utilization is impossible without solving the problem of liberating chlorides in the short-circuited /59
"human-human" cycle.

Chemoautotrophic organisms

Very serious attention, particularly by American authors, was given to the study of hydrogen-fixing bacteria Hydrogenomas eutropha. Even in 1965 at the Sixteenth Congress of the MAF*, Jenkins reported on the results of three-year studies on the possible use of hydrogen bacteria for regenerating oxygen from carbon dioxide in combination with water electrolysis. Schematically, these processes take place as follows:

Bonding of carbon dioxide occurs during heterotrophic synthesis:

$$2H_2 + CO_2 \longrightarrow (CH_2O) + H_2O \quad (1)$$

The energy which is necessary for the synthesis of organic matter is produced due to oxidation of hydrogen in the bacteria organism, with the formation of water

$$4H_2 + 2O_2 \longrightarrow 4\,H_2O \quad (2)$$

* International Astronautical Federation.

In sum, the chemical work of the hydrogen bacteria may be expressed by the equation

$$6H_2+2O_2 + CO_2 \rightarrow (CH_2O) + 5H_2O \quad (3)$$

If we add to this the results of the respiration process in the human organism

$$(CH_2O) + O_2 \rightarrow CO_2 + H_2O \quad (4)$$

then the sum of these processes (3 and 4) is equalized by the electrolysis of water

$$6H_2O \rightarrow 6\ H_2 + 3O_2$$

As a result, a system is obtained with a closed cycle with respect to oxygen, carbon dioxide, and water. /60

In the experiments of Mandel and Shapiro [121], three generations of rats received a diet consisting of 27 weight percent of bacteria biomass. No changes were observed in the females with respect to their fertilization, birth, and raising of offspring.

However, in later studies of Waslien et al. [138], it was found that the human organism poorly tolerates a biomass of hydrogen-fixing bacteria in the human diet.

Table 12, taken from this study, gives the symptoms and the time for their formation after receiving from 15 to 26 grams of dry biomass of hydrogen bacteria in four volunteers. Almost in all of them, dizziness, nausea, vomiting, and diarrhea occurred after each time they ate food including the bacteria.

Before 1968, a nutritive system based on hydrogen-fixing bacteria was regarded as very promising. However, in light of the data of the inapplicability of these bacteria as nutrient products for humans, their use has been essentially discarded.

Additional studies must be made to provide a final solution to the problem of the possible use of hydrogen-fixing bacteria, even in the form of a supplementary food source.

IV. Study of Experimental Models of Biological Life Support Systems

/61

A decisive stage in the study of BLSS is experimental modeling, which is necessary in order to study their functional characteristics, mechanisms by which stability is maintained and lost during prolonged operation, and the principles underlying control and regulation. With this research aim, the models studied must have the maximum amount of closure with respect to the material balance, even in the case of damage to their weight and energy characteristics, a certain amount of comfort for humans, and other characteristics which are important for real systems. Only with a maximum amount of mass exchange closure can the models studied illustrate their degree of stability, the basic functional and operational characteristics, and in the last analysis the possibility of creating a new class of life support systems on a certain scale.

Earlier studies on the simplest models of biological systems were primarily qualitative in nature, and contained no quantitative information regarding the metabolic cycle.

The first experiments studying the possible use of algae photosynthesis for providing gas exchange for animals were performed by American researchers just after the flights of the first artificial Earth satellites of the Earth and spacecraft [105, 110, 111, 124]. In 1961, in the School of Aviation Medicine (USA) experiments were performed with monkeys which lasted up to 50 hours [102].

In 1960 — 1961, together with the Institute of Plant Physiology of the USSR Academy of Sciences, our coworkers performed similar experiments on rats and dogs which lasted more than 170 hours, as well as two experiments with humans which lasted about one day each [32]. /62

In these experiments, flat containers were used which were illuminated from the outside by luminiscent lamps. The total amount of suspension was about 200 liters, with a culture density up to 3 grams per liter. The process took place in a stored culture, with limited illumination. The productivity of the reactors was insufficient to provide human gas exchange and this was the only reason for ending the experiments. After the hermetic cabin was opened, everyone, except for the test subjects, experienced a sharp unfavorable odor of algae. It is interesting that the test subjects concurred in this opinion only after taking several breaths outside of the chamber, and then again returned to it.

The next experiment with a human lasted longer (56 hours), and was carried out with the coworkers from the Boeing firm in 1962 [103]. In every case, the experiments were stopped due to insufficient productivity of the photosynthetic reactors, accompanied by accumulation of carbon dioxide in the system and a decrease in the oxygen content.

However, an important conclusion of these studies, which were started on animals and continued on humans, was that the direct combination of algae photosynthesis with human gas exchange is possible in principle. At the same time, many new problems arose, which indicated that even in such a simple system the relationships are not as elementary as was first believed.

Thus, in addition to the data obtained previously regarding the liberation of carbon monoxide by Chlorella [47, 102, 105, 110, 124, 128] it was found that Chlorella may absorb several gas admixtures from the atmosphere, including carbon monoxide [48]. With respect to the liberation of carbon monoxide by algae, it was found that the specific accumulation of this toxic component in a regenerated atmosphere has an inverse relationship to the photosynthesis rate, and thus is indirectly connected with the photosynthesis process. /63

Thus, for example, with a low suspension productivity (on the average 0.04 g/l·hr), the liberation of carbon monoxide is 2.07 ± 0.5 milligrams per one gram of dry algae material in the reactor. With a suspension productivity which is one order of magnitude greater (0.3 g/l·hr), the amount of carbon monoxide liberated per one gram of dry algae biomass is one order of magnitude less (0.09 ± 0.02 milligrams).

The assumption was advanced above that, in analogy with the formation of endogenic carbon monoxide in the human organism, its production during the cultivation of algae may be related with the oxidizing destruction of the tetrapyrrole ring in the chlorophyll molecule [47].

The inverse dependence of the carbon monoxide accumulation in a system on the photosynthesis rate was established in later studies of Kazakov et al. [33, 34] (see Figure 10).

The possibility of prolonged stable cultivation of algae in a closed system (see Section 3) has enabled us to again turn to the experimental study of the "man-algae" system, but on a more refined methodical basis.

At the present, we may speak of a new stage in the complex study of the simplest models of biological systems, including humans, with a gradually increasing complexity of the system structure and the closure of its mass exchange. The purpose of /64
this modeling is to study the interactions which actually build up between its functional links when they operate together in the entire system, which cannot be achieved by studying the properties of isolated links.

The first long experiment with a two-link "man-algae" system, whose gas exchange was closed, was carried out under the leadership of Professor I. Gitel'zon at the Institute of Physics of the Siberian Division of the USSR Academy of Sciences. It was continued for 30 days, and revealed the possibility of a prolonged, direct combination of human and Chlorella gas exchange [38]. This was then confirmed by the same authors in a sixty-day experiment with a three-link system, whose photoautotrophic link also included a hothouse with wheat, in addition to Chlorella [40]. The proportion of wheat plants in the total photosynthesis of the autotrophic link was about 18%.

In addition to establishing human gas exchange, these experiments also studied the possibility of the direct utilization of urine and everyday water in an algae reactor. The possibility of the prolonged introduction of urine into the algae

culture, without the accumulation of toxic concentrations of sodium chloride, was apparently provided in these experiments by a sufficient amount of water being able to flow through the algae cultivator (and the system as a whole). According to the data of the authors [39], the outflow of water from the system (for analyses, the loss of water in the feces and the raw biomass, etc.), which was compensated by water from sources outside of the system, was 5.3 liters per day, and the water requirements for feeding the algae by photosynthesis equalled 2.3 liters per day in total. Thus, the amount of water which passed through the system was 200% more than the water required in the system. /65

These experiments studied the way to obtain nutritive water. The condensate obtained at the output of the algae reactor was subjected to purification with the use of an oxidizing-catalytic method. Sanitary-chemical and bacteriological analyses as well as toxicological experiments on animals showed the suitability of the water obtained for food. In the second experiment, the condensate of water vapor from the atmosphere of the greenhouse in which the wheat was grown was used for this purpose also.

Thus, these experiments pointed to the potential possibility of closing the water balance in the system studied.

With respect to the practical implementation of this possibility, it is closely connected to the degree of system closure with respect to food, and the possibility of recovering water from all of the moisture-containing waste products, including the algae biomass, removed from the system and carrying water which was combined in the photosynthesis process.

With the maximum reduction of all water losses from a system, the specific degree of closure of the water balance in existing models with food reserves is limited by the unavoidable introduction into the system of chemically combined water, which is mobilized during the assimilation of nutritive matter in the human organism. Even when products containing no water are used, the amount of this so-called metabolic water is 350 grams/day in the regular diet. The same amount must be removed from the system, and this limits the possibility of complete closure of the water balance until the system is also closed in terms of food. In general, the balance of water, food, oxygen, and carbon dioxide have a quantitative interrelationship, since each one /66 contains elements (oxygen, hydrogen, and carbon) which are included in all of the other elements, and without closure of the system in terms of food, the system cannot be balanced in terms of elements with these substances.

We studied the possible degree of closure of the water balance in one of our experiments with a "man-Chlorella" system, which lasted for 15 days. In this experiment, water obtained from the condensate at the algae reactor output was the sole source of nutritive water for the test subject [97].

It was possible to decrease the amount of water introduced into the diet to 835 grams. This determined the water flow through the system, which was 32% of the water required by man (2590 grams/day). Consequently, the water balance closure in the system was 1 — 0.32 = 0.68 or 68%.

In this experiment, we did not use any methods of purifying the regenerated atmosphere of gaseous admixtures, which made it possible to study the dynamics of some of them in the system. In particular, it was found that in the "man-Chlorella" model

studied, there was accumulation in the atmosphere of carbon monoxide, with subsequent prolonged stabilization at a relatively constant level (Figure 11). This points to the presence of two opposing processes in the system: the liberation and combination of carbon monoxide, and the relationship between these processes determines the level of the equilibrium concentration of the given admixture in the atmosphere. A sudden increase in this level, with an unspecified increase in the acidity of the culture medium in the algae reactor, in 14 — 15 days points to the dependence of the equilibrium level on the state of the algae culture. /67

Figure 11 shows the dynamics of the methane content in the air system in the experiment. In this case, the content of the gas being studied stabilized only after 11 days. However, there was apparently some natural mechanism here, which limited the content of this component in the atmosphere.

Ammonia and hydrogen sulfide] were not detected in the atmosphere of the system throughout the entire experiment, including the last stage when up to 15 grams/day of ammonia, obtained by evaporation of urine, were introduced into the air circuit of one of the algae reactors. This points to the great possibilities of an algae system as a means for purifying the atmosphere from certain harmful admixtures. On the whole, the problem of toxic admixtures in the atmosphere of a closed autotrophic-heterotrophic system still requires further study, including a study of the mechanisms by which these substances are liberated and combined in different links of the system.

The satisfactory hermeticity of the system in this experiment made it possible to determine the disbalance between the gas exchange of algae and humans. It consisted of the gradual increase in the carbon dioxide content in the air (it was periodically

removed by means of sorbents) with the oxygen concentration remaining the same at the beginning and end of the experiment. The disbalance obtained was 7.5% (which is customary for such systems) of the human gas exchange with a regular diet and a nitrate source of nitrogen for the algae. This corresponded to a total increase in the carbon dioxide content of 383 liters throughout all the experiments.

The use of urea as the nitrogen source for the algae may decrease or eliminate this disbalance. In principle, as was already indicated by Myers [125], the independent maintenance of the gas balance is only possible in a system where a human being or any heterotrophic "population" of the system requires all of the autograph biomass reproduced in the system. /68

As is known, there are still only very few studies on complete models of biological systems, and they only pertain to the simplest models with a low degree of closure of the metabolic cycle. Nevertheless, it is of definite interest, because new problems are confronted. In particular, we are concerned with the problem of the methods and criteria for a comparative analysis of different models with a different functional structure, different spatial dimensions and amount of matter cycled, and a differing period of existence in the experiments.

In this connection, the necessity has arisen of developing the most general prerequisites, which are suitable both for developing experimental models of biological systems, and for the subsequent comparative analysis of their basic parameters and their ability to provide information in an experimental study.

We have already discussed one of them previously. This was the adequate degree of closure of a system mass exchange. With a small degree of closure, any substance may flow through the system, and the characteristics of its cycle cannot be established in the case of real closure. In order to establish the degree of closure for the cycle of any substance, we start only with its rate of consumption in the system (E) and the rate at which it flows through with incomplete closure (e), when the losses of the substance are compensated by its introduction from sources outside the system, or vice versa when the unavoidable introduction of material into the system (in the form of any reserves, for example, of food) requires the extraction of an equivalent amount from the system. Then the system closure coefficient with respect to the given substance (K) is determined by the expression: $K = I - e/E$. If we thus use all the basic fluxes of the substance, we can thus represent the entire degree of closure of the system material balance. /69

In connection with an estimate of the degree of closure of the system material balance, we can speak either about the closure of the biological cycle or about the "technological" closure. In the first case, the determination of closure, for example, of the water volume will include only the human water requirements for thirst and with food, as well as the combination of water in the photosynthesis process. In calculating the closure of the water balance (see above), we did not include the water consumption for toilet needs, which may change very greatly and is not strictly a biological requirement. Consequently, in the first case we obtain the characteristics of the biological cycle in the system. In the second case, which reflects the consumption of material for the toilet, household needs, etc., we obtain the technological characteristics of the system.

Another initial prerequisite for the successful development and analysis of models of biological systems is the necessity of an adequately high specific cycling rate of the basic materials in the system, which will provide the greatest amount of information from the models being studied. This led to the necessity of introducing the parameter of the initial amount of circulating material. It is important for the experimental /70
models that a decrease in the specific (required for one human being) amount of circulating material in the system causes an increase in the rate at which they circulate in the regeneration cycles, and in addition increases the resolving power and volume of information provided by the models when studying their dynamic characteristics with the same experiment duration.

In this case, the requirements on the dynamic parameters of the system are in contrast to the requirements on its experimental model, which is designed to study the functional characteristics of the object being modeled.

We have already pointed out the unavoidably high rates at which matter circulates in artificial systems as a factor which decreases the dynamic stability of the system [95]. Nevertheless, the necessity of obtaining the maximum amount of information on the dynamic properties of the regenerated systems in a period of time which is as small as possible compels us to prefer the less stable models which provide the greatest amount of information. The "intensification" effect which arises in such models produces favorable possibilities for determining the phenomena in the system which can lie outside of the range of the resolving power of the research methods in the case of models which are compiled with natural spatial dimensions. This holds true, for example, for all phenomena related to the dynamics of microadmixtures in the atmosphere or water, or to the dynamics of microelements.

Finally, the third prerequisite pertains to determining the time characteristics of processes taking place in the models. The attempt to make a comparative analysis of experimental models /71
whose structure differs in terms of their absolute period of existence has not revealed the possibility of determining any general characteristics of models being compared. For this purpose, it is necessary to use some other units of time which are organically related to the properties of the system itself. As such a unit of time, we have considered the index of the natural time of the system as a segment of time in which one cycle of any material is completed. The natural time of a system in terms of the cycle of the given material (τ) is determined as the ratio of the amount of the given material in the system (Q) to the rate at which it is liberated or consumed (q).

For example, the air of a hermetic chamber with a volume of 20 cubic meters at normal atmospheric pressure contains 1000 liters of oxygen, which is consumed by man, and is regenerated in the system at a rate of 500 liters per day. Then the natural time of the system in terms of the oxygen cycle is 1000:500 = 2 days. Thus, this index characterizes the system only in terms of the rate at which any material is circulated. A comparison of the natural time with respect to the basic material flows makes it possible to establish the critical paths of the metabolic cycle as paths with the greatest circulation rate and, consequently, the least stability.

Figure 12 shows that, due to the closed nature of the system, the initial replacement of the initial atmosphere by the regenerated atmosphere follows an exponential law. In this connection, in practice the time required to replace the initial atmosphere (by approximately 95%) is three times greater than the natural time of the system. In essence, it is only from this moment /72

that the system being studied begins to exist. The nonstationary process taking place before this makes it impossible to establish the characteristics of the system being studied in terms of the metabolic cycle or, for example, the accumulation of volatile admixtures in the atmosphere.

One consequence of these prerequisites which is important in practice is that the use of a model in an experiment, whose duration is three times less than the natural time in terms of the basic material flows, is not of scientific interest. With a net experiment time less than 50% of its total duration, it will be less economical in terms of the labor consumption and amortization of the experimental equipment.

Thus, an important generalized characteristic of models being studied is the natural time of the system (τ), which includes the characteristics of the mass of circulating matter, for a specific concentration of this matter and the spatial dimensions of the system. From this index we may obtain the derivative indices which are suitable for a comparative analysis of the amount of material provided by models of bioregenerative systems:

— the duration of the nonstationary period ($3 \cdot \tau$)

— the "net" operational time of the system: ($T - 3\tau$) (the total duration of the system operation minus the duration of the nonstationary period)

— the number of "net" regeneration cycles of the given material: $(T - 3 \cdot \tau)/\tau$.

F

These criteria make it possible to formulate different models of biological systems with a different functional structure, mass /73
of circulating material and spatial characteristics, in comparable quantities. Table 13 gives a comparison of three models having different parameters which have been studied up to the present. Each of them provided the gas exchange for one human being. The data in Table 13 were calculated for the oxygen regeneration cycle, and may only be evaluated from this point of view.

The air volume of models 2 and 3 equalled, respectively, 12 and 5 cubic meters. The volume of the first model was calculated indirectly, with allowance for the wheat planting area, and the gas exchange of the test subject was calculated from the composition of the assimilated food diet, since there was no direct information on these parameters in existing publications.

At first glance, a paradoxical conclusion follows from the analysis of the data in Table 13: in terms of units of the system natural time, the shortest living model is model 1, which existed in the experiment for 60 days, and the greatest time of existence for model 3 in the experiment was 15 days.

However, if the unit of effective operation of a system, existing in a specific space with a specific amount of regenerated material, is assumed to be one regeneration cycle of this material (in this case oxygen), then in actuality model 3 existed seven times longer than the "net" operational regeneration cycles as compared with the model 1 and gave much greater information regarding the functional characteristics.

The comparative analysis given in Table 13 shows that the assumed generalized parameters may be used as unique "similarity criteria" when comparing models whose structure and spatial parameters differ, and also when providing a basis for the duration of experiments with these models. /74

The parameter of the natural time may be used in a theoretical analysis of a biological system stability by means of mathematical models of the material fluxes.

V. Conclusion

Thus, the studies on developing biological life support systems have led to the following basic results:

— The development has essentially been completed of methods for prolonged intensive culture of unicellular algae as applied to the conditions for a system with a closed metabolic cycle;

— The link of higher forms of plants approximates this stage of development;

— The development of methods for mineralization and return to the cycle of the products of human vital activity and other organic waste products still is far from complete. The basic limitation in this region is the accumulation in the system of chlorides and other components of human excretion, which are not required by plants. In addition, the problem of mineralization of organic waste products cannot be separated from their utilization in the system. Therefore, a final solution will only be possible when mineralized organic waste products are included in the system of the mineral supply for algae and higher forms

of plants, and also with the utilization of parts of the organic waste products in the intermediate link of heterotrophic organisms.

The development of a link of heterotrophic organisms has /75
essentially gone no further than preliminary determinations and certain preliminary experiments and tentative calculations of the possibility for using certain domestic animals and poultry.

Thus, the basic result of the previous period of research on BLSS is the development of methods for prolonged, intensive cultivation of the lower and higher forms of plants with recirculation of air and the water medium, i.e., with respect to the conditions and the requirements of a closed system.

This has made it possible, without waiting for the complete development of links of heterotrophic organisms and mineralization of organic waste products, to pass through a new stage of research — the study of the properties of experimental models of the simplest biological systems, including man, subsequently making more complex their structure and the degree of closure of the metabolic cycle as missing links are developed.

At the same time, a study of even the simplest BLSS models raises new questions and problems on improving existing functional links. The first cycle of research on the "link-system-link" thus seems to be completed.

The future course of BLSS research also appears to be cyclical: individual functional links will be perfected on the basis of studying models of entire systems, and they will provide the basis for the creation of perfected models and so on, until an adequately stable stucture and degree of closure for the metabolic cycle in a system is obtained.

As this complex and long process takes place, a very /76
important role will be played by mathematical methods in future BLSS research. Many of the data compiled up to the present have become the subject of a mathematical analysis, primarily aimed at a complex evaluation of conditions and effectiveness of algae cultivation, as well as optimization of the parameters of algae cultivators [20, 86, 94,a, 98a]. The greastest amount of material is generalized in the study of Smirnov [86a].

A very necessary, although much more complex, problem is the mathematical modeling of biological systems as artificial biogeocenoses. The search for optimum variations of BLSS structure and methods for controlling them only by means of natural experiments is not very realistic in terms of the material expenditure and necessary development time.

The studies of Watt [136b], and Svirezhev and Yelizarov [82] shed some light on this problem. They provide a generalization of the great amount of data on mathematical modeling of natural systems (populations and biocenoses). The second study deals with a subject which of greater interest to us, and contains original solutions which approximate these subjects.

Mathematical modeling is extremely important for analyzing the stability of biological systems. This unique method makes this analysis precede the creation of natural models. The study [82] contains different examples of the approaches to this problem based on an analysis of classical models of the Volterra type, at the basis of which lies a study of the number of species, as the main characteristic of the system state.

There have been much fewer attempts to model biological systems based on an analysis of material and energy fluxes. Among /77 these, we may point out the studies of Vinberg, Rubin et al. [16, 17, 79, 80].

It is possible that mass exchange models may be of particular interest in analyzing the stability of artificial biological systems, whose limits of stability are greatly determined by the limited nature of the space they occupy and the amount of circulating matter, i.e., the low damping capacity of the medium with respect to fluctuations in the matter flow.

On the first stages of this analysis, it is sufficient to clarify and study only those material flows, which are limiting paths in the network of material relationships between system components, both biotic and abiotic. For man, who is the most sensitive biocenosis component in the BLSS, the flows of oxygen and carbon dioxide will be the limiting paths. For plants, the limiting path may be water exchange, keeping in mind the role of water as the basic factor in the mechanism of plant heat transfer with high intensities of the light flux in a dense culture. The necessity of modeling and studying other paths — for example, human food relationships — disappears when establishing the instability of a system based on these indices. The malfunction of a link in terms of this parameter takes place 3 — 4 times more slowly than with respect to the gas exchange parameter. Based on this, mass exchange models — at least at the first stages of studying BLSS stability — may have an advantage over classical models of the number of populations and biogeocenoses.

One important intermediate result of the previous stage of research is the great reliability of biological systems as compared with nonbiological systems. The inadequate reliability of technical equipment used in the biosystems makes it impossible to establish much larger limits of reliability for the biological objects and processes. In any case, the previously expressed fears regarding the low genetic stability of systems based on the cultivation of rapidly multiplying micro-organisms have not been corroborated by many years of using one and the same strains in the biological system models studied. The theoretical bases for these fears were also weak, since in intense cultivators with continuous harvesting of crops, the possibility of mutations arising is in contrast to the possibility of removing them with the harvested crop and the influence of stabilizing harvesting under standard cultivation conditions. /78

This was shown on a mathematical model and by experimental means by Tsoglin, and was confirmed in a study of Shvytov [94b].

In conclusion, we would like to examine some secondary aspects of the development of BLSS, which are related to their possible use in science and industry.

We have in mind the possible use of intense and economical technological cultivations of different unicellular algae for producing protein and vitamin products for use in animal husbandry and poultry raising, as well as for partially solving the problem of protein in the human diet, taking into account demographic prognoses.

The development of an intensive, readily automated technology for cultivating higher forms of plants may be used to organize /79
the year-round industrial production of vegetables in the immediate vicinity of large cities, or in regions with climatic-geographical conditions which are unsuitable for this purpose. This technology may have an important influence when it is used to develop new types of agriculturally useful plants, whose selection periods may be accelerated by a factor of 4 and more.

The problems of cosmonautics have stimulated research on biotechnology to such an extent that much less effort is involved in adapting the results obtained to the needs of industry.

There is also no question of the general scientific importance of correctly formulated studies on BLSS. In terms of its scientific content, the creation of BLSS as a practical problem is no different than the experimental biogeocenology of artificial (i.e., anthropogenic) biogeocenoses. Larger and larger regions of land and water with their agricultural areas, pastures, and piscicultural and hunting economies are becoming such biogeocenoses. Biological systems, in the sense employed here, represent the limiting cases of such systems, and therefore are acceptable models for a precise quantitative study of processes to be modeled, when their mass exchange is sufficiently closed.

The value of such models for a study of real natural systems may be seen in the example mentioned earlier of the accumulation of chlorides in water tanks which receive sewage. In experimental models, this process extending over several years /80
was reproduced experimentally for several weeks.

Experimental-ecological studies of models of biological life support systems with a relatively closed metabolic cycle may be of interest for studying the very pressing problem of the action of man on the biosphere.

The development of biological life support systems has up to the present only been "nourished" by the achievements of other branches of science and industry. There is more and more basis for assuming that the opposite process will be accelerated as this problem is studied further.

TABLE 1.

BASIC PARAMETERS OF BIOLOGICAL METABOLIC CYCLE IN THE EARTH BIOSPHERE[1] *

Components of biosphere	Absolute amount, tons	Per unit of biomass, ton/ton	Production or requirement, ton/year	Duration of regeneration cycle, years
Phytomass (dry material)	$2,4 \cdot 10^{12}$	-	$2,32 \cdot 10^{11}$	10
Ocean phytomass	$1,7 \cdot 10^{8}$	-	$6,0 \cdot 10^{10}$	$3 \cdot 10^{-3}$
Ocean water	$1,37 \cdot 10^{18}$	$5,7 \cdot 10^{5}$	-	$1 \cdot 10^{6}$
Atmosphere	$5,1 \cdot 10^{15}$	$2,1 \cdot 10^{3}$	-	-
Atmos. oxygen	$1,18 \cdot 10^{15}$	$4,9 \cdot 10^{2}$	$2,32 \cdot 10^{11}$	$5,1 \cdot 10^{3}$
Atmos. carbon dioxide	$2,35 \cdot 10^{12}$	1,02	$3,4 \cdot 10^{11}$	6,9

[1] The table is compiled from the data in the paper of N. Vazilevich et al. [8].

*Commas represent decimal points.

TABLE 2.

BASIC PARAMETERS OF BIOLOGICAL METABOLIC CYCLE IN REGIONS WITH THE HIGHEST CONCENTRATION OF LIVING MATTER (TROPICAL FORESTS)[1]*

Components of biosphere	Absolute amount, tons	Per unit of biomass, ton/ton	Production or requirement, ton/year	Duration of regeneration cycle, years
Biomass (dry material)	$1{,}17 \cdot 10^{12}$	–	$7{,}7 \cdot 10^{10}$	15
Atmosphere	$1 \cdot 10^{14}$	$8{,}8 \cdot 10^{1}$	–	–
O_2 in atmosphere	$2{,}3 \cdot 10^{13}$	$1{,}97 \cdot 10^{1}$	$7{,}73 \cdot 10^{10}$	$3 \cdot 10^{2}$
CO_2 in atmosphere	$4{,}7 \cdot 10^{10}$	$4 \cdot 10^{-2}$	$1{,}14 \cdot 10^{11}$	$4 \cdot 10^{-1}$

[1]The table is compiled from the data in the paper of N. Vazilevich et al. [8].

*Commas represent decimal points.

TABLE 3.

POSSIBLE PARAMETERS OF BIOLOGICAL LIFE SUPPORT SYSTEM FOR A FIVE-MAN CREW*

Components of biosphere	Absolute amount, tons	Per unit of biomass, ton/ton	Production or requirement, ton/year	Duration of regeneration cycle, years
Total biomass (dry weight)	$1 \cdot 10^{-1}$	–	–	–
Atmosphere	$6{,}5 \cdot 10^{-1}$	6,5	–	–
O_2 of atmosphere	$1{,}4 \cdot 10^{-1}$	1,4	1,5	$1 \cdot 10^{-1}$
CO_2 of atmosphere	$1{,}8 \cdot 10^{-3}$	$1{,}8 \cdot 10$	1,8	$1 \cdot 10^{-3}$
Water	1	$1 \cdot 10^{1}$	7,3	$1{,}4 \cdot 10^{-1}$

*Commas represent decimal points.

TABLE 4.

THE EXTENT OF WORK DONE ON VARIOUS BIOSYSTEMS, AS APPLIED TO SPACE CONDITIONS[1]

Biosystem	Gas exchange	Utilization of wastes	Full value of products
Algae	4	2 (3)	2
Higher plants	2 (3)	I	I (2)
Yeasts and molds	0	I	0
Animals	0	0	0

0 = lack of data, or the data do not apply to space conditions

4 = sufficient amount of data

[1]The table is prepared on the basis of data from NASA Ames Research Center [101].

TABLE 5.

YIELD OF ELEMENTS FROM NUTRIENT MEDIUM PER 1 g OF THE BIOMASS PRODUCED

Elements	Authors		
	Lebedeva[1] et al. [51] mg/g	Kuznetsov[2] et al. [49] % with respect to nitrogen	Gitel'zon[3] et al. [21] mg/g
Nitrogen	82±I,9	I00%	90
Phosphorus	I7,6±I,7	I8	II–I2
Sulfur	II,2	5,9	4–5
Potassium	I6,0	I9	I0–II
Magnesium	5,7±I,2	5,5	3–4
Iron	0,3	–	–

[1]Chl. pyrenoidosa Source of nitrogen - nitrate
[2]Chl. pyrenoidosa Source of nitrogen - urea
[3]Chl. vulgaris Source of nitrogen - urea

TABLE 6.

COMPARISON OF THE CHEMICAL COMPOSITION OF CHLORELLA GROWN UNDER DIFFERENT CONDITIONS WITH A HUMAN DIET[1]

Material	Content of material in % of organic mass		
	Proteins	Carbohydrates	Lipids
Human diet	16	69	15
Biomass of Chlorella:			
at temp. 37°C	55	13	32
at temp. 43°C	20	50	30

[1]Table taken from the work of V. Semenenko et al. [85].

TABLE 7.

COMPOSITION OF THE BIOMASS OF VARIOUS FORMS OF UNICELLULAR ALGAE[1]

Form of algae	% per dry weight			
	Proteins	Fats	Carbohydrates	Ash
Chlorella Sp. K	52	20	23	5
Anacystis hidulans	82	3	I0	5
Chl. reinhardii -449	3I	2I	4I	6
Spirulina platensis	64	II	I2	I3

[1]Taken from the work [98].

TABLE 8.

VARIANTS OF SPECIES STRUCTURE IN PLANTINGS OF HIGHER PLANTS[1]*

Plant species	Planting area, m^2	
	Variant 1 [Ref. 30]	Variant 2 [Ref. 93]
Potato	II,2	-
Head cabbage	I,5	I,I
Leaf cabbage	0,4	-
Carrot	0,6	0,6
Radishes	0,3	-
Table beetroot	0,7	0,3
Tomatoe	I,6	-
Rice	-	I0,7
Sweet potato	-	2,I
Dill	-	0,2

[1]From the data of IMBP MZ SSSR [30, 93].
*Commas represent decimal points.

TABLE 9.

COMPOSITION OF NUTRIENT SOLUTIONS FOR HIGHER PLANTS*

Component	Amount g/liter				
	1	2	3	4	5
Potassium nitrate	1,10	0,70	1,00	-	-
Calcium nitrate	-	-	0,40	1,29	0,43
Ammonium nitrate	-	-	0,10	-	-
Potassium phosphate (monosubstituted)	-	-	0,25	0,75	0,25
Calcium biphosphate	0,31	0,30	-	-	-
Potassium sulfate	-	-	0,63	0,63	0,10
Calcium sulfate	0,76	0,60	-	-	-
Magnesium sulfate	0,52	0,54	0,20	0,50	0,17
Ammonium sulfate	0,14	0,12	-	-	-
Total salts	2,85	2,25	1,95	3,17	0,95

1 and 2 - from data of the U.S. Department of Agriculture [137]
3 - recommended by Prof. B. Chesnokov for hydroponic growth of fruit cultures
4 - as applied in IMBP MZ SSSR for the growth of potatoes under conditions of substrate hydroponics
5 - as applied at the same place for aeroponic growth of potatoes.

*Commas represent decimal points.

TABLE 10.

PRODUCTIVITY OF CERTAIN FRUIT PLANTS UNDER CONDITIONS OF LIGHT-CULTURE[1]

Plant	Vegetation period, days	Yield g/m^2 day					
		Total		Net output		Waste	
		wet	dry	wet	dry	wet	dry
Head cabbage	90	264	24	I55	I5	I09	9
Leaf cabbage	40	4I2	24	375	2I	37	4
Carrot	90	266	34	I78	20	88	I4
Table beetroot	90	380	48	220	3I	I60	I7
Tomato	90	340	30	220	I3	I20	I7
Radish	40	I65	II	I50	9	I5	2
Potato	90	I33	3I	III	28	22	3

[1]Data from the Institute of Medico-Biological Problems, MZ SSSR.

TABLE 11.

RATE OF PHOTOSYNTHESIS AND RESPIRATION OF HEAD CABBAGE AT VARIOUS OXYGEN CONTENTS IN THE AIR [DATA FROM REF. 69]

Content of oxygen in air, %%	Day of experiment	Absorption of CO_2 in 18 light hours, g	Lib. of CO_2 in 6 dark hours	
			in g	in % with respect to absorption
20-22 (Control)	I	I3,42	0,6I	4,5
	2	I4,26	0,62	4,3
	3	I3,95	0,62	4,4
	4	I4,08	0,45	×
	5	I3,99	0,59	4,2
	6	I4,I4	0,67	4,6
	Average	I3,97	0,62	4,4
24-26 (Experiment)	7	8,62	0,83	9,6
	8	8,89	0,84	9,4
	9	9,23	0,87	9,4
	I0	9,50	0,85	9,0
	II	7,66	0,89	II,6
	I2	8,88	0,8I	9,I
	I3	8,40	0,75	8,9
	Average	8,74	0,83	9,5
Difference		-5,23	+0,2I	+5,I

Experimental conditions: planting area 25 dm^2; area of leaves, 62-73 dm^2. Intensity of illumination, 100 W/m^2; temperature 18-21°C; CO_2 content in the air, 0.3 - 0.35 %.

× no reading on respiration was taken, because of a sharp decrease in temperature in the dark period.

TABLE 12.

REACTION OF THE HUMAN ORGANISM TO THE ADDITION TO FOOD OF AUTOTROPHIC HYDROGEN-FIXING BACTERIA HYDROGENOMONAS EUTROPHA*

Test subject	Time of intake, hours				Time of appearance of first symptoms of:				Weight of excrements, g/day
	08.30	I2.30	I7.30	2I.30	Dizziness	Nausea	Vomiting	Diarrhea	
A	8,6	I7,2	-	-	No	I0.00	I3.00	I5.00	Not recorded
B	8,6	6,6	-	-	09.30	I0.00	I5.00	I3.I5	92I
V	-	-	9,6	5,I	I9.00	I9.30	No	22.30	423
G	-	-	I7,2	-	I9,30	20.30	2I.00	at night	I265
D	-	-	-	-	No	No	No	No	0
E	-	-	-	-	No	No	No	No	277

D and E - Control test subjects.
Data taken from the work of Waslien et al. [138].
*Commas represent decimal points.

TABLE 13.

COMPARISON OF CHARACTERISTICS OF GENERALIZED PARAMETERS OF VARIOUS MODELS OF A BIOLOGICAL LIFE SUPPORT SYSTEM

Model	Experiment duration, days T	Nat'l time, days τ	Transition process 3τ	Net time of exp. $T-3\tau$	Net cycles of regeneration $\frac{T-3\tau}{\tau}$	Experiment duration in units of nat'l time $\frac{T}{\tau}$
1	60	17	51	9	0.5	3.5
2	30	6.7	20	10	1.5	4.5
3	15	2.2	6.6	8	3.6	6.8

× 1 - The system "man-algae-wheat" [40]

2 - The system "man-algae" [38, 39]

3 - The system "man-algae" [97].

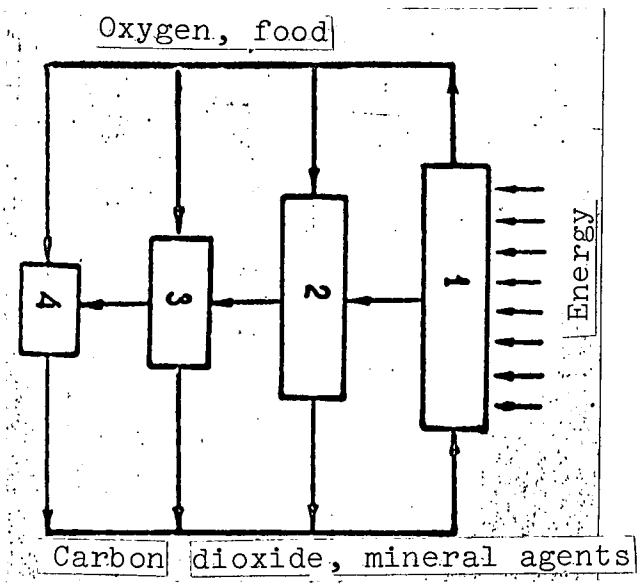

Figure 1. Line drawing of trophic links in biological systems.
1 — Photoautotrophs; 2 — Herbivores; 3 — Carnivores;
4 — Microorganisms - mineralizers

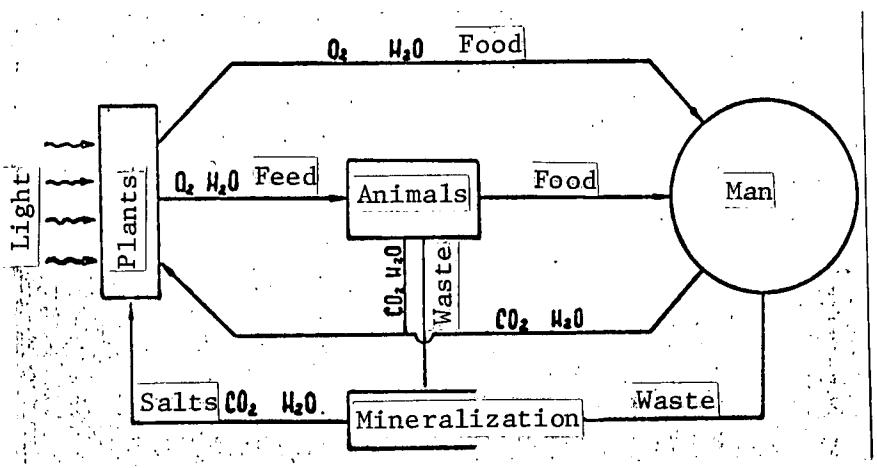

Figure 2. Line drawing of a biological life support system.

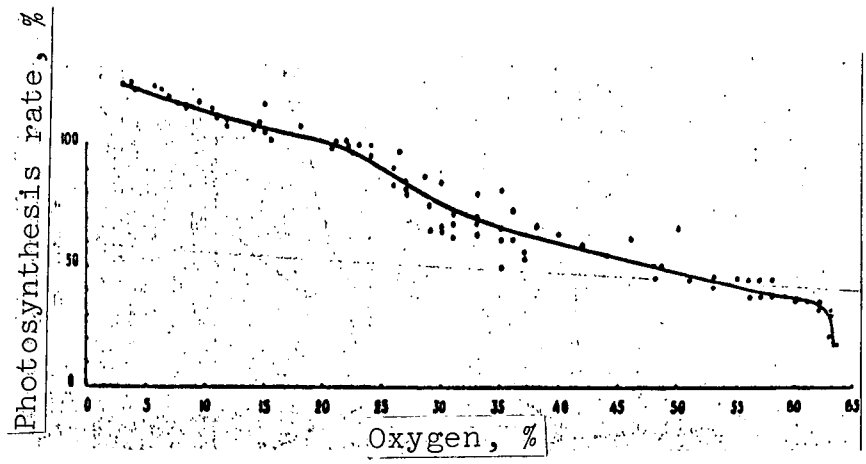

Figure 3. Photosynthesis rate in a syspension of Chlorella at various concentrations of oxygen in the air.*

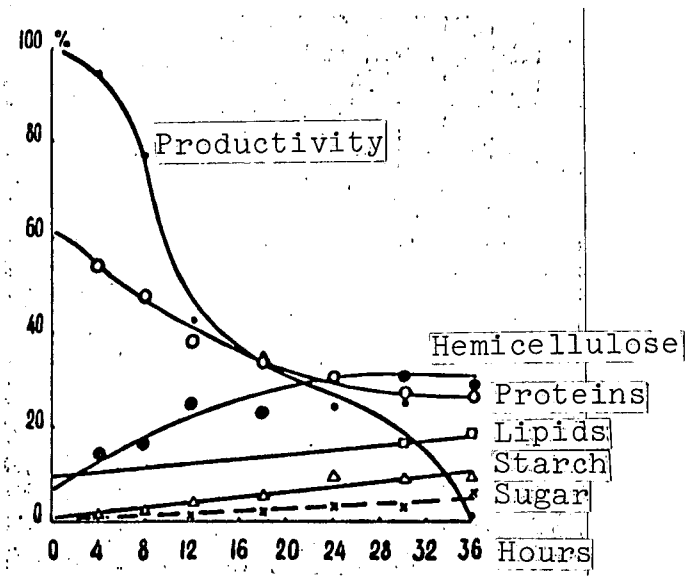

Figure 4. Change of productivity and content of organic matter in Chlorella with no nitrogen in nutrient medium (in % of the initial content in the normal nitrogen diet).**

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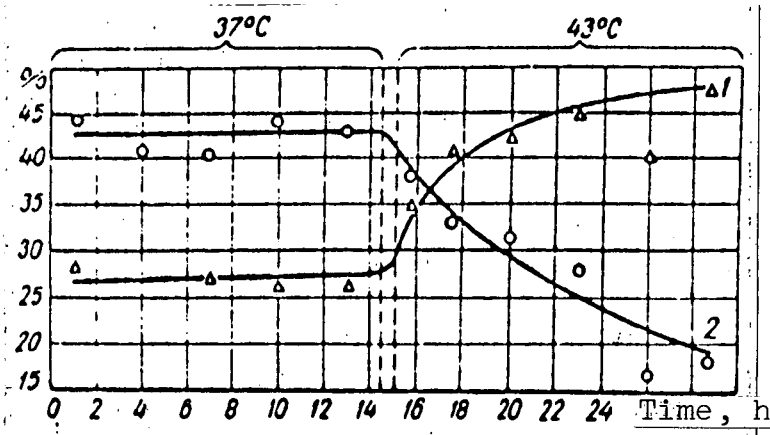
* From the paper of G. Meleshko, et al. [66-a].

** Compiled from data given by Sadikova et al. [83].



Change of the Chlorella chemical composition (1 - carbohydrates; 2 - proteins) when cultivation temperature is changed.

Figure 5. Change in the content of carbohydrates (1) and proteins (2) in Chlorella biomass with increased temperature of the medium.*

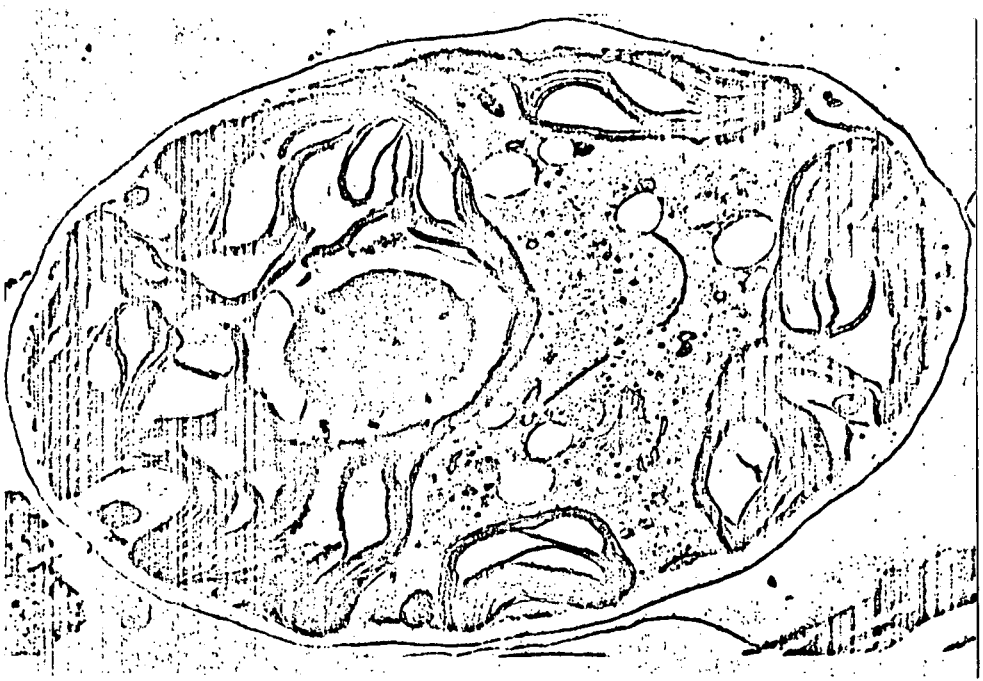


Figure 6. Reserve food material stored in cells of elamidomonad (magnification 4500X).

* From the paper of V. Semanenko et al. [85].

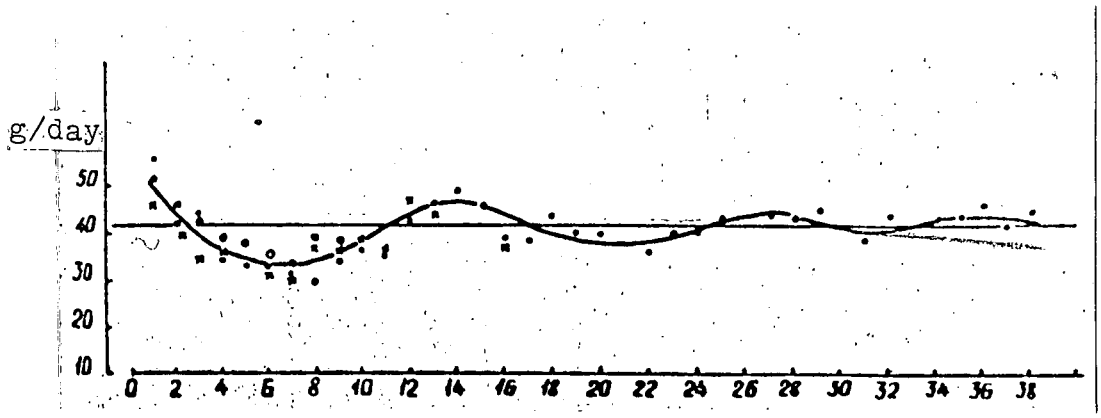


Figure 7. Fluctuations of the productivity of Chlorella suspension cultivated under standard conditions.

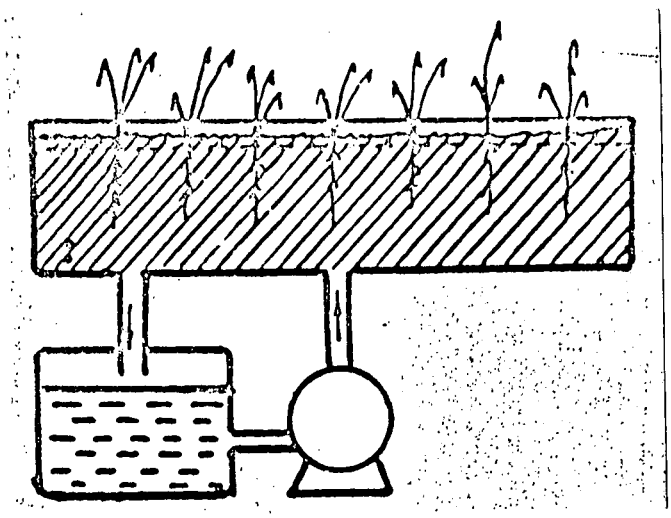


Figure 8. Line drawing of a device for hydroponic growing of plants. H - pump.*

* From the work of V. Dadykin [30].

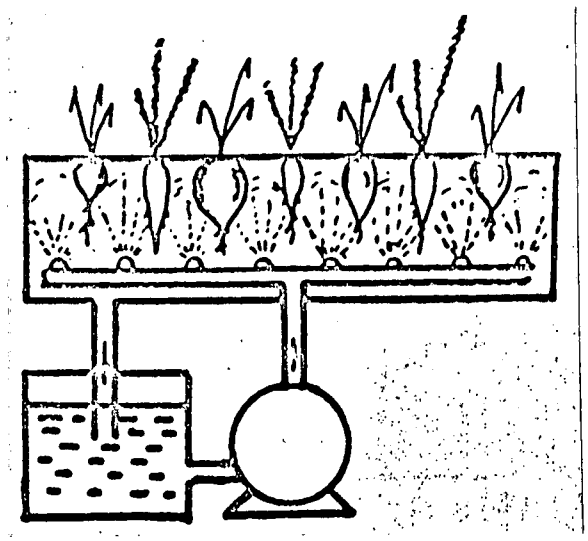


Figure 9. Line drawing of a device for aeroponic growing of plants. H - pump.*

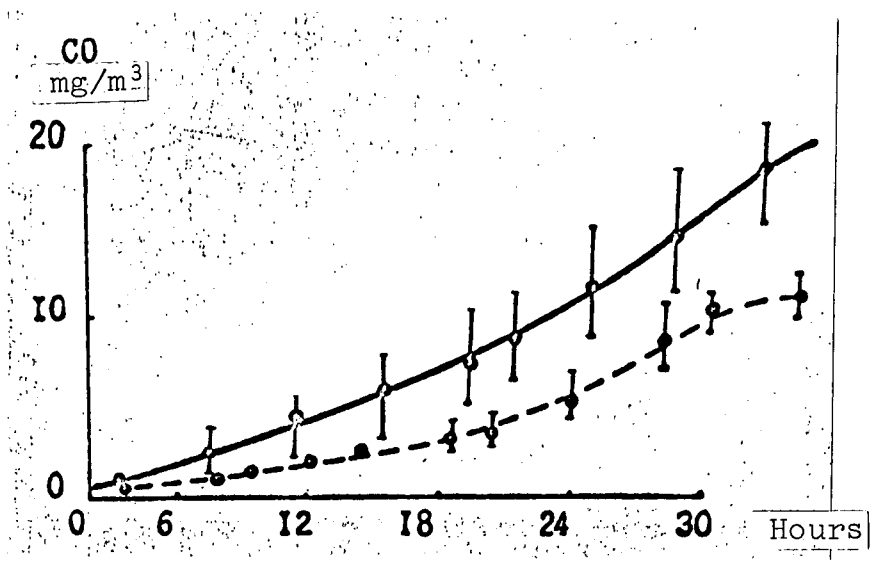


Figure 10. Accumulation of carbon monoxide in the atmosphere of an algae gas exchanger at different photosynthesis rates: curve 1 - 0.5 l CO_2 per 1 l suspension per hour; curve 2 - 1.0 l CO_2 per 1 l suspension per hour.**

* From the work of V. Dadykin [30].

** From the paper of A. Kazakov, et al. [33].

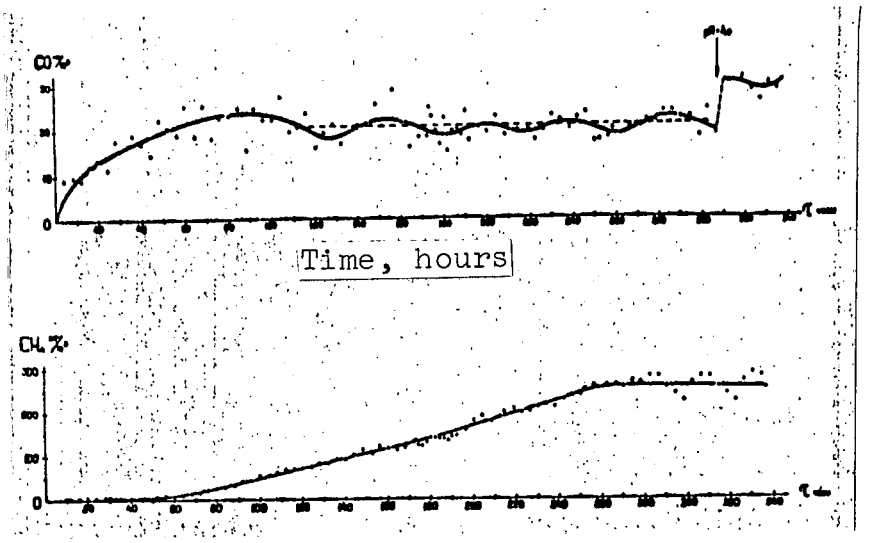


Figure 11. Content of carbon monoxide (upper curve) and methane (lower curve) in atmosphere of the "man - Chlorella" system which is closed with respect to gas exchange.*

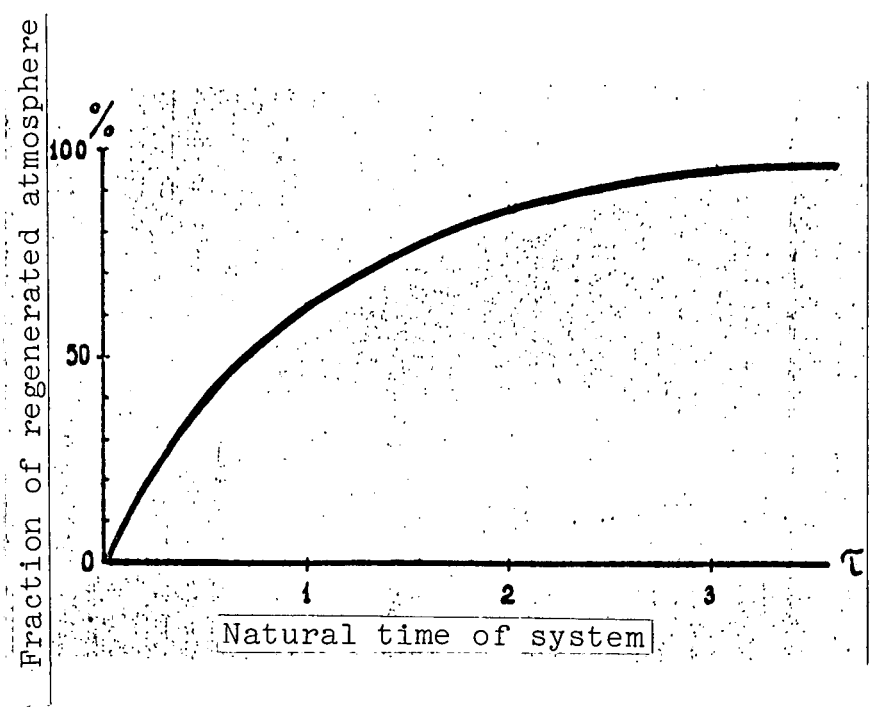


Figure 12. Dynamics of the replacement of initial atmosphere by regenerated atmosphere in a closed system (in units of natural time).

* From the data of IMBP [97].

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